

Secrecy beyond reason

The Reagan Administration continues to apply widely its demand for secrecy in scientific matters that have any bearing on national security. It is time for more vocal opposition.

CAN only two years have passed since officials of the Reagan Administration were visiting scientific meetings and warning American researchers that academic science was "the soft underbelly" of national security? In 1982, the administration's apparent obsession with the notion that Soviet spies were tricking American scientists into giving away the nation's technological secrets set the government and the universities at loggerheads. In several celebrated cases, the universities (notably and creditably Stanford University in California) refused State Department requests to act as policemen when Soviet and Chinese scholars visited their campuses. To defuse the situation, the National Academy of Sciences published a report, *Scientific Communication and National Security*, which tried to find a reasonable compromise between the legitimate worries of the defence establishment and the traditional commitment of the United States to free and open communication in science. By 1984, technology transfer — at least insofar as it affects the universities — appears to have become the great forgotten issue of US science policy.

A famous victory for the National Academy of Sciences? Hardly. If little is heard about technology transfer, it is not because the Reagan Administration has promptly accepted the recommendations put forward by the academy's report. In a choice irony, the deafening silence from the White House is a product of a decision that the administration's response to the academy's report on scientific communication should itself be kept secret. Dr Frank Press, the academy's president, originally expected an early response from the White House science office. Later, the job was handed from the science office to a senior inter-agency group meeting under the aegis of the National Security Council. Now, the response is believed to be complete but it has been classified as secret.

It would be tempting to believe that there was little cause for concern. After all, most recent cases of government intervention in technology transfer have consisted of efforts to prevent Eastern bloc nations from illegally importing bits of American hardware, such as sophisticated computers. It was always the contention of the universities that technological transfers of that kind — rather than international communication in academic science — were the only really significant examples of hostile countries gaining militarily useful information. The National Academy panel, chaired by Cornell University's former president, Dale Corson, concluded that academic science was a relatively unimportant source of Soviet poaching, and that the dispute between the universities and the administration could be resolved by building tall fences around a very small number of areas of great sensitivity. The universities have been as good as their word; their voluntary agreement to submit papers on cryptography for pre-publication screening has, for example, worked remarkably well.

There is however, little reason to suppose that the administration has decided to accept the general philosophy of the Corson report. The available evidence suggests the opposite. Far from agreeing to confine its attention to a small number of highly sensitive technologies, the administration appears to want to extend its control over scientific communication as far as possible. In a little-noticed recent directive, for example, the Department of Defense has begun to insist that all basic and applied contract research it sponsors in universities must be submitted for pre-

publication screening. Only if the research cannot be placed within a very loose definition of "sensitivity" can it be submitted for publication without pre-screening.

It is too early to say whether this new policy will result in the suppression of articles that could otherwise be published without compromising national security. One hopeful sign is that the Department of Defense has at last set up an internal appeals board to review borderline decisions. But there are also ominous signs that the department's aggressive attitude towards technology transfer is fostering something akin to a siege mentality in which relatively low-ranking officers are opting for secrecy simply because they want to play safe or, in some cases, because they do not understand the complex rules through which the administration's technology transfer policy is being implemented. Recent meetings on materials science, held at the University of Dayton, Ohio, and the University of California, Los Angeles, have been closed to foreign nationals, including scientists from North Atlantic Treaty Organization (NATO) countries. And last month a meeting of the American Ceramics Society in Cocoa Beach, Florida, was split into two halves with one, technically no longer a meeting of the society, being sponsored separately by the Department of Defense and the National Aeronautical and Space Administration and restricted to US citizens.

The prevalence of secrecy in government departments which sponsor scientific research is bad enough. What is worse is the apparent unwillingness of academic scientists to kick up a fuss when unwarranted restrictions are placed on publications and meetings. In 1982, it was the readiness of scientists to insist on the importance of open scientific communication that prompted the National Academy to produce the Corson report. Now that the report appears to have vanished without trace, the need for vigilance is all the greater. □

Time to back Europe

When research ministers meet in Brussels next week, they should back the Commission.

THE French launched the idea of "a European 'space' for science and technology" in their all-but forgotten memorandum to the Athens summit last December. In Toulouse a couple of weeks ago, socialists from the European Parliament backed the same idea. The meeting was unfocused and hence ineffectual, but at least it indicated concern: Europe is certainly not a "space for science", but a polyglot and divided continent where scientific mobility is almost non-existent.

Something must be done! The cry has echoed around the laboratories of Europe for years — but now there is a new determination to find structures that will work, to avoid institutional atrophy and tired bureaucracy. The goal is real, flexible, European collaboration in science and technology.

Even that temple of bureaucracy and powerhouse of paper in Brussels — the European Commission, the executive arm of the European Economic Community (EEC) — is fired with the new zeal. It burns brightest in the person of research and industry commissioner (and vice-president) Etienne Davignon, a Belgian tipped for the presidency next year, and in his director-general for research, Italian biologist Professor Paolo Fasella. With the aid



of their enthusiastic staff, they aim to revitalize research in Europe and to prepare the ground for European industries based on the new technologies. That is why, next week, when European research ministers meet in Brussels to discuss Commission proposals, they should have the courage to back them without regard to the suffocating weight of the "budget problem".

The research ministers, who meet for no more than a few hours a year on European issues, must be prepared to delegate some responsibility — and call for European cooperation. The Brussels hierarchy for science has never been in a better state than now to accept the responsibility. Moreover, the Brussels research budget is tiny, less than two per cent of what is spent on research and development by the ten member states, and only a tiny fraction of the total Brussels budget (two-thirds of which goes to support over-productive European agriculture). A little courage to back excellence, in this situation, would go a long way to remind the world — and Europeans — that Europe can be a constructive as well as a destructive union.

Admittedly, Brussels-backed research has had its faults. Even the new \$1,000 million information technology research programme, ESPRIT, a key paper on the ministers' table next Tuesday, is not faultless. (It could pay greater attention to training.) The Commission also has a legacy of *ad hoc* research programmes dating from old post-war treaties, and a sometimes burdensome in-house research centre at Ispra near Milan. But almost everything that can humanly be done with this legacy seems to be being done. Last year council approved the "framework programme", which for the first time relates Brussels research support to a list of political priorities, and moves as far as possible towards a conceptual and practical unity for the work. More imaginatively, the Commission launched CODEST, a small group of illustrious technocrats which aims to spend where the money will get greatest leverage: in part, in creating mobility across almost impenetrable European borders and in part on an eight-laboratory collaboration in optoelectronics. All well and good. But when the Commission asked for more cash, the Council of Ministers only managed to approve the principle.

Another item on next week's agenda is biotechnology, where the Commission has proposed a large-scale programme that faces several Community issues (such as feedstock prices). The Commission does not dare hope for approval, but the faintest nod would be encouraging. And the Council should remember a previous far-sighted biotechnology, first put on the table many years ago, which took literally years to approve. The programme, much trimmed, now goes under the title of "biomolecular engineering" and by all accounts is a great success.

What if the ministers fail to provide backing? Suppose they are so frozen in Europe's recrystallizing nationalism that they can offer Messrs Davignon and Fasella — and Europe — no life-line. Is that the end of European scientific collaboration, and so of any hope of a European high-tech reply to Japan and the United States? No, because frustration is bound to spill over into some solution whatever happens to the EEC.

An example is a body called CERFACS, still a dream at present. This proposed "European centre for advanced research and training in computer sciences" is the result of an astronomer's holiday in the Pyrenees, when Dr Jean-Pierre Poyet of the Observatoire du Pic du Midi near Toulouse met a member of the European Movement (out of which the idea for EEC originally emerged). The European Movement in Toulouse, still rather active, was seeking European projects for the region, and Poyet — frustrated by the death of expertise in vectorial computing in Europe (the kind that needs Crays) — invented CERFACS, which would fill the gap. That was a years ago. The idea has been enthusiastically adopted in France, by researchers and industry alike, but if it shows signs of becoming a reality Poyet would like to see it avoid the EEC maelstrom, by becoming a multilateral project. Even the European Parliament appears to agree: it has given money for a feasibility study. The moral: if research ministers refuse to back imagination in Brussels, there is yet hope that it will emerge elsewhere. □

Supercomputermania

Japanese advances should be welcomed by scientists, not treated as national threats.

THE argument of last resort in US science policy debates is inevitably the invocation of the Soviet peril. If that fails, the Japanese peril will do. It is never a very intelligent way to carry on a debate, but in an administration that feels threatened by the Soviets' use of vacuum tubes in their fighter planes and in a country that has been shaken to the core by Japan's ability to build cars that do not fall apart, it hits home. Thus the news that Fujitsu is soon to produce a supercomputer to rival those made in the United States seems to be doing more to excite interest in supercomputing than all past arguments about the urgent need for more supercomputing facilities for US researchers.

This need was detailed most recently by a working group set up by the National Science Board, the governing body of the National Science Foundation (NSF). The working group recommended that NSF commit hundreds of millions of dollars over the next few years to the establishment of academic supercomputer centres to fill the void left by current facilities, which are either in industry (the oil companies, for example, are very interested in numerical modelling of petroleum reservoirs) or the national weapons laboratories (which are very interested, still, in what happens when a thermonuclear bomb goes off). Meanwhile, astrophysicists and chemical engineers, who could benefit from large-scale numerical modelling, have nowhere to go.

The arguments failed to carry the day in this year's budget proposals. NSF is still apparently reluctant to get back into the business of supporting mainframe computer centres, having abandoned that policy years ago in favour of supporting the purchase of laboratory-sized mini- and microcomputers. Most campus-wide computer centres have fallen into disrepute among scientific researchers — whether as a cause or effect of this policy shift it is not clear. Into the breach have fallen the research projects that demand a massive number-crunching capability.

At least NSF director Edward Knapp has been able to announce a modest programme to support the use of existing supercomputers by researchers — but not without invoking the Japanese peril. And there was the start last week of what will surely become a major stir when reports came trickling in from US scientists who had seen prototypes of Fujitsu's new machine, with *The New York Times* printing what is coming to be the standard "Japan outstrips the West" story.

Yet, in reality, the Japanese development is good news for science everywhere. Whether or not the Fujitsu is faster than US machines is not the point. What the Japanese are commendably doing is to develop the software that will make these machines appealing to business as well as scientific users — the Fujitsu will reportedly be compatible with IBM software. Ninety per cent of supercomputer costs are at present spent in developing software peculiar to non-standard machines. If the Japanese can change that, they will sell a good many more supercomputers, which can only bring the price down and allow more scientists access to them. If supercomputers are brought within the reach of more people, more uses will be developed for them. That will be good news to the computer industry everywhere — even to IBM. □

Nature in Tokyo

Nature has opened an office in Tokyo. Dr Alun Anderson, for the past few years in charge of the News and Views section of Nature, will be in charge of the office for an initial period of one year. By this means, Nature hopes more efficiently to cover news of developments in Japan and also to provide tangible help to Japanese scientists wishing to submit research papers or other communications to Nature.

Dr Anderson's address is *Nature*, Macmillan Shuppan KK, Eikow Building, 10-9, Hongo 1-chome, Bunkyo-ku, Tokyo 113 (Telephone (03) 816 3756/7; Telex JATOK J32121 MSKK). □

Illmensee inquiry

Fraud charge unproven, researcher resumes duties

THE commission set up by the University of Geneva to investigate allegations of fraud against a leading embryologist, Professor Karl Illmensee, has concluded that there is no compelling evidence that he fabricated his data. But in a report that is highly critical of Illmensee's experimental records, the six members of the commission are unable to reach agreement on whether "the hypothesis that the protocols were fabricated could be upheld"; some felt it could, others considered "there was no compelling evidence to support or refute the hypothesis". Releasing the full report last week, the university blandly stated that Professor Illmensee is resuming all his duties in the faculty.

The commission had been considering since last August allegations made against Illmensee by three members of his laboratory. The allegations centre on a series of experiments that Illmensee carried out in Geneva between April and August 1982. Growing suspicious of their professor, three members of the laboratory compiled evidence of fraud until, after a talk by Illmensee in January 1983, Dr Kurt Bürki publicly stated that he and his colleagues could not accept the results. An internal investigation of their accusations culminated in what some took to be an admission of fraud by Illmensee (who has since consistently denied it). The rector of the university then publicly announced that the affair would be investigated by a commission which included Dr Anne McLaren of University College London, Professor Pierre Chambon of the University of Strasbourg and professor Richard Gardner of the University of Oxford.

Their report concentrates on three matters — Illmensee's apparent admission of fraud, the numerous errors, discrepancies and corrections discovered in his experimental protocols and errors in a grant application he submitted to the US National Institutes of Health (NIH).

Illmensee's admission amounted to a signed statement that "Protocols of experiments of Dr Karl Illmensee have been manipulated in a way which is contrary to scientific ethics in some period of 1982". It was countersigned by three members of the faculty of sciences of the University of Geneva and dated 17 May 1983. But what exactly did it mean? The faculty members, in a letter that accompanied the statement when it was sent to the dean, said that "Illmensee clearly recognized having falsified ("faked") protocols. . .", a view which they subsequently maintained under questioning by the commission. Professor Illmensee, however, maintained throughout the commission's enquiries and last

week that he had admitted only errors in writing up his protocols, not falsification.

Speaking from Geneva, Illmensee said he was so shocked at the accusations made at his meeting with the faculty members that he could not defend himself properly and allowed words to be put in his mouth. Faced with contradictory statements, the commission was finally unable to decide whether Illmensee "did or did not recognize by his signature that experiments were falsified".

It was the day after he signed the admission that Illmensee wrote to NIH withdrawing a sentence, concerned with a particular chimaeric mouse, from his grant application. Faced with contrary evidence, Illmensee acknowledged that the mouse could not be the product of the experiments he had claimed it to be in the application. Subsequently he corrected his "slip" but with a claim that has since become as doubtful as the first. The report concludes: "At the present time, the Commission, as well as Professor Illmensee, do not know which mouse, if any, was the chimaeric male referred to in the NIH grant application." Last week Illmensee disagreed, saying that the words "of two" should replace "if any" since somebody other than himself must have mistakenly mixed up two animals.

As to the accumulation of errors, corrections and discrepancies in his records which, according to the report, "are such as to throw grave doubt on the validity of the conclusions" of the series of experiments, Illmensee admits that his recording system was too prone to error and says that a contributory factor was the stress caused by his impending move to new buildings. He emphasized the report's conclusion that the errors and discrepancies discovered do not bias the results claimed.

Responding to criticism by the commission of his secretive way of working and failure to share techniques with his colleagues, Illmensee said this was exaggerated. He had shared each part of the technique with some colleagues although not every step could be carried out by anyone in his laboratory and certainly not with the level of success he could achieve. The techniques are highly skilled and "it takes Ashkenazy to play Rachmininov properly" said Illmensee.

The report does not reserve its criticism for Illmensee. It is also critical of the university for not making Dr Bürki's written accusations available to Illmensee for several months even though they had been given to other members of the university staff. Moreover, it criticizes those members of the university who felt

concerned about the case for not analysing the Bürki report in sufficient detail before drawing their conclusions and not discussing it with Illmensee.

As for Dr Bürki and the two other accusers, the commission acknowledges their sincerity but is mildly critical "of their failure rigorously to assess their evidence" and for their lack of "on-the-spot documentation to support their accusations", because lack of such documentation complicated the commission's task.

Professor Illmensee expressed satisfaction with the way the commission worked (he was allowed to attend all hearings with his lawyer) and with the result. He will now have to reconstruct his laboratory — the accusers and their sympathizers will be leaving — and attempt to repeat the dubious experiments as a collaborative project with full scientific rigour, as suggested by the commission. So far he has no definite plans for that and also has to await the reaction of NIH to his grant application and the Swiss National Fund's decision on a grant that was blocked half way through its 3-year term, pending the outcome of the commission.

Dr Bürki, who did not receive a copy of the report in advance of its publication, said last week that he accepts the report as it is and was glad that the scientific community would be able to read it for themselves. (More than 700 pages of transcripts from the commission's hearings are also open to anyone who wishes to travel to Geneva.)

Peter Newmark

Orlov appeal

Dr Yuri Orlov, the Russian physicist and human rights campaigner, who completed a seven year prison-camp sentence on 10 February (see *Nature* 16 February, p.585), is to serve the second part of his sentence, five years' internal exile, in the Yakut Autonomous Republic, one of the bleakest areas in Siberia.

The decision to send Dr Orlov to Yakutia was taken on 6 February — three days before the death of Mr Andropov. Mr John Macdonald, the London lawyer who has been a noted campaigner for Dr Orlov's release, has therefore made a new appeal to Mr Chernenko "to make a gesture towards the scientific community throughout the world and release Orlov".

To show Dr Orlov that his colleagues abroad have not forgotten him, the members of the "Orlov Committee" at the European Organization for Nuclear Research (CERN) this week applied for a visa for one of their number to visit Orlov in exile, although they realize that the chances of obtaining it are small. More practically, they are organizing their colleagues to send greetings cards and scientific journals to Orlov. The following address should be sufficient: Dr Orlov, Yu. F. Yakutskaya ASSR, Leninskii Raion, Poselok Nurbachan, USSR. Vera Rich

US biotechnology

Information instead of products

Fort Lauderdale, Florida

THE biotechnology industry has been given warning that in the absence of products or revenue it must keep the investment community up to date with as much information as possible on product development, marketing strategies and other internal management and financial plans.

"Uncertainty is the bane of existence for the stock market", said Nelson Schneider of E.F. Hutton at a meeting here sponsored by the Industrial Biotechnology Association to bring together its member companies and representatives of the investment community. Schneider and other stock analysts at the meeting noted that with so many venture capital companies going public, Wall Street finds it difficult enough as it is to evaluate their worth and that conventional methods could easily conclude that many of the biotechnology stocks are in fact worthless.

Robert Johnston of Johnston Associates pointed out that valuations based on such traditional methods as comparisons with other stocks are of little value in biotechnology, where expectations, supply and demand of the stocks and the "greater fool theory" — the belief that a stock is a bargain because someone else is willing to pay more — operate.

Robert Fildes, president of Cetus, retorted that the new biotechnology companies were being asked by the analysts to "take down their trousers", while the pharmaceutical companies' privacy was respected. And he dismissed the analysts' arguments

expertise, quality control, packaging and management are crucial elements that many of the new biotechnology companies are only beginning to assemble.

The hazards of going public, however, are sufficient to stop some, at least for the time being. Gabriel Schmergel, president of the Genetics Institute, said his company would remain private for the "foreseeable future". He was conscious of the pressures of going public, one of which is the very uncertainty the market feels for the industry. "The long-term nature of our business is not fully appreciated by the public", he said. Fluctuations in the value of stock on the market can be particularly unnerving, he said, both for management and for the staff who own stock.

A number of biotechnology companies, including Genex, Agrigenetics and Genentech, have resorted recently to commercial bank loans to support their operations, which Schneider said may indicate "a useful new movement".

Stephen Budiansky

Nuclear Pakistan

'Islamic bomb' scare resurfaces

THE vexed question of Pakistan's nuclear capability has surfaced again, first in rumours widely reported last month in the Asian press, but since strongly denied, that China had supplied Pakistan with nuclear bombs and second through a published interview with a leading Pakistani nuclear scientist, Dr Abdul Qadeer Khan, director of the Kahuta uranium enrichment plant.

In this interview, which appeared first in the newspaper *Nawa-e Waqt*, and was repeated next day on Lahore radio, Dr Khan said that he felt "cornered" by questions about a possible Pakistani bomb, and did not know whether to answer yes or no. This remark immediately produced a counterblast from Soviet television which alleged that Dr Khan had boasted that it was within Pakistan's capability to create its own bomb. (The Soviet commentator incidentally identified Dr Abdul Qadeer Khan as head of the Pakistani Atomic Energy Commission — a

Harvard's Ptashne patent disappoints

Washington

HARVARD University is embarrassed in its efforts to sell the rights to a fundamental genetic engineering process developed by Professor Mark Ptashne, a Harvard faculty member. After approaching hundreds of companies, Harvard has succeeded in interesting fewer than a half dozen in purchasing a licence to the pair of patents covering Ptashne's invention.

Harvard was trying to duplicate Stanford University's success with the Cohen-Boyer genetic engineering patent, which currently has 68 licensees, each paying a fee of \$10,000 a year against future royalty payments of ½ per cent of net sales. The Ptashne patent covers another fundamental step in genetic engineering: the method for attaching a bacterial promoter to a gene of non-bacterial origin, such as that coding for human growth hormone. Harvard, which also wanted \$10,000 a year and ½ per cent royalty on sales, apparently misread both the importance of the Ptashne patents and the mood of the industry. Ironically, Genetics Institute, the Boston-based biotechnology company that Ptashne founded and with which he remains closely associated, did not even take out a licence; its president, Gabriel Schmergel, said that Harvard's terms were "onerous" and that "alternative routes to achieve the same end result" of the Ptashne process are available.

Albert Halluin, patent counsel for Cetus Corporation, attributed the poor response to the reluctance biotechnology companies to head down a path of a whole series of

basic process patents, each taking a ½ per cent bite out of sales. And he says that the companies have become much more knowledgeable about patents. "After the history of the Cohen-Boyer patent" — the validity of which has come under considerable question since the companies first signed up — "people are saying maybe we should take a hard look at these. Now people have learned that any patent may have flaws if you look hard enough."

Halluin also noted that many companies took licences to the Cohen-Boyer patent at least partly to show that they were "one of the club" of new biotechnology companies. On that basis, and with a cut-price initial licensing offer, Stanford collected more than \$3 million from licensees even before the first products were sold. Harvard also announced that stiffer licensing terms would operate after termination of the initial offering — at the end of 1983.

Schmergel cited another reason behind his company's decision: he said Genetics Institute was "upset" by what he termed Harvard's preferential treatment in the granting of exclusive licences on some of its other biotechnology patents to companies founded by the original inventors of the patents — in particular Dr Walter Gilbert of Biogen and Dr Max Essex of Cambridge Biosystems.

Schmergel said Genetics Institute had discussed with Harvard the possibility of obtaining an exclusive licence on the Ptashne patent but had been unable to reach an agreement.

Stephen Budiansky

"I think I've come up with the answer to the world's food problem, but I'm still stuck on the packaging...."



that the pharmaceutical companies can be rated by their track record and product stream: "What you're saying is that they have a bigger war-chest than we do", he said, "but they'll go under too, eventually, if they don't produce".

Many of the industry's representatives noted that going public is an essential step in raising the funds needed to become a business selling products rather than being merely a research and development firm. Hugh D'Andrade of Schering-Plough said that his company has a sales force of over 5,000. "It's more than brilliant scientists who turn discoveries into products." D'Andrade said that manufacturing

post actually held by Dr Munir Ahmed Khan, who claims to have little to do with his namesake from Kahuta.)

Dr Abdul Qadeer Khan, in fact, has stressed the entirely peaceful intentions of the Pakistani nuclear programme. He said that unlike India, where the nuclear power programme had been "based from the very beginning on sound foundations" under the patronage of the late Pandit Jawaharlal Nehru, Pakistan had made a relatively late and initially ill-organized start. Moreover, India, at least initially, could get encouragement and help from developed countries, but fear of an "Islamic bomb", which could be disseminated to all Muslim countries, had led the developed countries to impose restrictions on the export to Pakistan of even "the most minor products".

A bomb-making capacity depends, ultimately, on a uranium enrichment facility. On this point Dr Khan did not explicitly commit himself. He claimed only that Pakistan was far in advance of India in uranium enrichment and "not far behind foreign experts" in any field. The Pakistani Government is at present, he said, trying to purchase a 900 MW light-water reactor, and the uranium processing plant at Kahuta was being used to produce the necessary fuel.

Since it would take at least 10 years to obtain, install and operate such a reactor, the Atomic Energy Commission was "positive" that the fuel supply would be ready when needed. Those who thought that the plant was being used for uranium enrichment were deluding themselves, he said. A few minutes later, however, he observed that "as far as the supply of enriched uranium is concerned, we shall supply the Atomic Energy Commission, God willing, with as much as it needs."

Had they come from a politician, Dr Khan's words might well, for reasons of national prestige, be trying to imply a self-sufficiency which has not yet been attained. Dr Khan, however, is a scientist, and moreover, something of a special case. He was sent to study in Europe and then worked at the Almelo uranium enrichment centre in the Netherlands, returning to Pakistan in December 1975. He was subsequently charged, *in absentia*, by the Dutch government with industrial espionage, and he was sentenced, still *in absentia*, to four years imprisonment — a verdict which for prestige reasons, the Pakistan Government is still trying to get reversed.

As Dr Khan admitted in his interview, a special Dutch parliamentary committee appointed to investigate the affair stressed that Pakistan had gained much capability and saved considerable research expenditure on uranium enrichment as a result of his activities — although he maintains that the Dutch judgment was "unjust", "contrary to all the canons of law" and inspired by "anti-Pakistan and anti-Islam" elements.

Vera Rich

Nuclear power

French, plentiful and cheap

THE French national electricity utility EDF is making money at last, despite the enormous cost of the French nuclear power programme, which left EDF with debts of FF 150,000 million (£12,500 million). So claimed EDF director-general M. Jean Guilhamon during a visit to London, in a talk orchestrated by Sir Walter Marshall, chairman of the British Central Electricity Generating Board. Sir Walter, despairing of the future of nuclear power in the United Kingdom, used the opportunity of Guilhamon's visit to sign a fast breeder agreement (*Nature* 9 February, p.495) to ask him to demonstrate that in France, at least, nuclear power works. M. Guilhamon kindly obliged.

The giant EDF debt — in order of magnitude not far from the national debt of Brazil — has been amplified over the past two years by the halving of the value of the French franc against the US dollar — in which currency EDF borrowed much of its capital. But even allowing for making repayments and interest at commercial rates and for depreciation of plant, EDF is now making a profit, says Guilhamon.

France now produces 54 per cent of its electric power by nuclear plant (mostly pressurized water reactors), and with 26 more reactors already under construction or on order, the figure will be 70 per cent by 1990. That is near the practical maximum, given that it is difficult to switch off nuclear power to cope with nightly falls in demand — although EDF is offering customers cheap rates if they will take power nearly continuously.

Opponents of nuclear power have condemned the cost of building the stations, but the tables are now turning, Guilhamon claims. During the construction programme, the government set a low electricity price which made EDF unprofitable, he says. But the price encouraged new electricity consumers: for example, two-thirds of housing starts in France are now all-electric. Now, as more and more nuclear power stations turn on, displacing costly oil- and coal-fired plant, the profits of the programme are beginning to appear.

"In 1976 we were burning 14 million tonnes of oil. In 1984 we will burn less than 2 million tonnes", said Guilhamon. Coal burning has also fallen from 18 million tonnes to 11–12 million tonnes. "So we are now getting a very good return on our investment" at electricity prices at around 30 per cent below those of other countries in Europe.

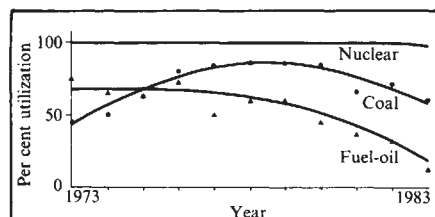
Guilhamon compared the whole French nuclear power programme, which by 1990 will amount to some 50,000 MW and spare France the purchase of some 50–60 million tonnes of oil, with the scale of British North Sea oil production.

Sir Walter Marshall, meanwhile, openly feared the loss of key British industries to

French soil, when in the 1990s French commercial electricity could cost only half the price of British power. Imperial Chemical Industries (Britain's biggest chemicals company) and British Steel might decamp to France, Marshall suggested.

Meanwhile, however, EDF does have a few problems caused by over-capacity and strong political pressure to burn French coal, and so keep the French miners happy. The surplus to traditional French markets will reach 50,000 million kWh in 1990, according to EDF, or around 15 per cent of total production. EDF will seek to place 20,000 million kWh in new markets it hopes to create in industry (for example, electricity to power boilers) and to export the other 30,000 million kWh. Certainly, at present French prices, electricity exports are increasing: 13,000 million kWh last year compared with 4,000 million kWh in 1982.

The pressure from coal, however, may be more difficult to withstand. Last week the government was attempting to arbitrate



Utilization of the average net operating power available from France's power stations. Coal and oil powered plants are used well below their capacity. The 100 per cent figure for nuclear power plants hides the loss in capacity due to structural defects and engineering problems.

between the French mining company Charbonnage de France (CdF) and EDF over a dispute in which CdF insists that EDF must buy fixed amounts of coal, or pay a fixed amount of money to CdF, for the next four years. EDF calculates this would cost it FF 2,500 million (£200 million) a year, essentially to support CdF. EDF does not wish to pay the bill (and since both companies are nationalized, the transfer of funds is somewhat notional); but it may have to.

Robert Walgate

● Meanwhile, accountants commissioned in 1982 by the then British energy secretary, Mr Nigel Lawson, to look into CEBG's pricing policy have argued forcefully that British electricity was not only over-priced (Sir Walter Marshall would agree with that — the government has set the price too high, he says) but ineptly priced (a comment less palatable at CEBG). The complex calculations by which the CEBG set its rate to bulk industrial customers are not based on sound economics, the accountants say. A proper pricing formula would result in cuts of 5–10 per cent followed by a few years' freeze. □

West German universities

Minister responds to Knopp

LAST week, West German Minister of Education, Dr Dorothee Wilms, announced her intention to recast the federal university law in line with the recent Knopp report (see *nature* 26 January, p.305). The existing law, the 1976 *Hochschulrahmengesetz* which was designed to provide a unifying framework within which the *Länder* could develop their own legislation, was brought in by a social democratic (SDP) government. Now in opposition, SDP finds the commission asked the wrong questions and came up with the wrong answers. "Partly useless, partly unnecessary, and partly reactionary", commented Eckart Kuhlwein, SDP spokesman.

On the other hand, the West German Rectors' Conference (WDRK), representing the heads of universities, agrees with the report that modest change is needed to create more flexibility within which the *Länder* can act, especially in the areas of personnel, career grades and financing from sources outside the universities.

Controversy still centres on the power of the professors — of feudal proportions before the 1976 law. The report suggests strengthening faculty representation and ensuring professorial majorities in decision-making processes. Both the government and WDRK favour this change. The vice-president of the Free Uni-

versity of Berlin, however, has pointed out that this is tantamount to a return to hierarchy and less equality, towards scientific freedom away from communal control in the coordination of teaching and research. He points out that the universities could not have met the challenge of increasing student numbers without the middle grades of academic staff, which the report suggests should in future assume a still lower position relative to the professors.

A recent conference at the Berlin Aspen Institute pinpointed the failure of West Germany's universities to produce the quantity and quality of trained people the country needs. Large numbers of students, straitened finances, long periods of study, and centralized administrations have created a system in which it is often impossible for the gifted student to distinguish himself. Employment bottlenecks on the academic ladder make it difficult to maintain the pool of academic talent. One highly controversial remedy was presented by the Free Democratic leader and Foreign Secretary Hans-Dietrich Genscher, who restated his party's support for private universities and institutes. A positive comment on the private university at Wittne/Herdecke suggests that the Christian Democratic administration warms to this idea. Others point out that private can mean second-rate.

Policy formation for West German higher education is inscrutable to the point of opacity, emerging from a fog of relationships between the *Länder* and federal institutions and a series of quasi-official advisory bodies, including WDRK, the Wissenschaftsrat, the Conference of Ministers of Culture and the Federal-Land Commission for Educational Planning. These bodies are criticized for being outside parliamentary control, for their obscure nomination procedures, for overworking senior personnel, for consensus policies, and for a general lack of democratic legitimacy and openness.

This system will produce the new *Hochschulrahmengesetz*, which must also be seen as one facet of the administration's overall drive for industrial growth based on science and technology. **Sarah Tooze**

Italy's biotechnology

FAST action

Milan

ALTHOUGH basic biotechnological research is good in Italy, the same cannot be said of its application in industry. Thus concludes a report on Italian biotechnology published by the Federation of Scientific and Technical Associations (FAST), which calls for the establishment of a study committee to decide on some priority applications.

That call was endorsed by Luigi Granelli, Minister of Research, in welcoming the report. At last, he said, Italy has an exhaustive survey of the state of the art of biotechnology in the 150 Italian institutes where relevant research is carried out. The FAST report, added Granelli, is a realistic guide to combining public and industrial efforts in biotechnology, particularly at a time when Trieste is likely to be chosen as one of the two sites of the United Nations Industrial Development Organization's International Centre for Genetic Engineering and Biotechnology (see *Nature* 16 February, p.583).

The report was edited by Professor Gabriele Milanese of the Biochemical Genetics Institute in Pavia. The message of the survey is that basic research in biology in Italy compares well with other European countries, thanks to recent university law and to the projects supported by the Consiglio Nazionale delle Ricerche, Italy's principal research council, in continuance of the policy implemented since the 1950s by the late Professor Adriano Buzzati-Traverso. By contrast, industrial applications are poor despite the involvement of three public companies (Farmitalia-Carlo Erba of the Montedison group, Assoreni and Sclavo of the Eni group) and five private companies (Sorin Biomedica of the Fiat group, Lepetit, Recordati, Soreno Institute and Societa Produzione Antibiotici) and despite the fact that industry cooperates with public centres by investing in basic research.

Paola De Paoli

China

Storm over beancurds

AN attempt by the director of a Chinese research institute to add "technological pilferage" to the list of ideological shortcomings against which there is a current drive in the country has backfired. As a result, Ge Fengheng, director of the Liaoning Provincial Commercial Science Research Institute, has found himself the object of a campaign against "local monarchs in science" that may be widely applied.

The incident, known as the "beancurd storm", began last October when an engineer from the Liaoning Institute, Wu Wentao, read to a meeting on food additives a report about a new variety of "liquid beancurd" he had developed. This evoked widespread interest, since beancurd, a staple protein source, is in short supply in many places in China, and the new product could do much to fill the gap. A special meeting of the institute's academic council was accordingly convened, at which director Ge congratulated Wu and immediately accused him of "pilferage". According to Ge, Wu had presented his work without asking the institute or reporting to it afterwards, worked on a project not assigned by the institute, included foreign data in his report, and placed special emphasis on his use of additives to distinguish between his beancurd and other types already in pro-

duction.

The Liaoning Commercial Department ordered an investigation, and on 7 December reported that the production of such beancurd was unnecessary. By this time the distinguished journal *Shipin Gexue* (Food Science) had published Wu's preliminary research report. This apparently brought the matter to the attention of the party newspaper, the *People's Daily*, which discovered that during the past few years, Ge had repeatedly criticized the work of the institute's researchers as "pilferage", forbidding them to discuss the line their research should take, and used his position and power to penalize those who showed signs of opposing him. As a result, *People's Daily* concluded, there had been "serious losses" to the prestige of the party and to the "party's cause of scientific research".

The "beancurd storm", says *People's Daily*, has implications far beyond Liaoning, raising the issue of the personal qualities needed in the head of a research institute. Beijing radio went even further: "local monarchs" like Ge would not have been able to function were there not also "mistakes and a bureaucratic style of work" in the bodies responsible for administering the country's science. **Vera Rich**

Satellite launchers

Ariane backers confident

Evry, France

IN France, where they build Ariane, Europe's answer to the US space shuttle, one newspaper last week reckoned the score in own goals to be: Shuttle 3, Ariane 2. That is, the shuttle has lost one more communications satellite than Ariane, although it has regained one too. Certainly this leaves the space insurance business equally nervous of both systems.

But at Evry, south of Paris, from where the French space agency CNES and the commercial launcher company Arianespace run the Ariane programme, there is an increasing sense that Ariane may prove to be a winner in the billion-dollar market for communications satellites.

Without any sense of crowing over shuttle problems — for Ariane has had its own, and may yet have more — it is pointed out at Evry that launch from the shuttle into geostationary orbit is more complicated and more expensive than launch by Ariane. A communications customer on the shuttle must also buy a substantial rocket — the "perigee motor" — to lift the satellite from the low shuttle orbit to the elliptical geostationary transfer orbit (GTO), which reaches up to the parking height of geostationary satellites. It is these motors that failed in the recent shuttle flight. Ariane reaches GTO in one shot, using liquid fuel stages which are theoretically more accurate and reliable.

However, even if there is some delay in the shuttle's geostationary launch programme, Arianespace is not in a position to gain any immediate benefit. Its order book is almost full for two years, with room (perhaps) for only one more "good" customer in 1985.

The limiting factor is the Ariane production line, which can turn out no more than six launchers a year. From 1985, the launch site at Hourou, French Guiana, will be able to cope with 12 launches a year. So Arianespace, which is desperately undercapitalized (it could be bankrupted by a single launch failure), is seeking new capital in Europe to construct a second production line.

In the long run, however, much depends on the Ariane development programme. So far, Ariane launches have used only the first version of Ariane. A week ago, the last tests were completed on the second and third versions (Ariane-2 and Ariane-3, identical except that the latter has two extra strap-on solid boosters in the first stage). The first new version launched will be an Ariane-3, due to take the European Space Agency's ECS-1 and the French Telecom 1-A aloft this July. To maintain confidence in Ariane, of course, the new rocket should not suffer the teething troubles of Ariane-1 — which suffered high frequency vibrations in the first stage (causing failure of the second launch test) and then lubrication failure in the upper stage cryogenic fuel

pump (that knocked out the fifth). According to the man in the hot seat, the *Chef de Projet* for Ariane-3 (and a future Ariane-4), André Van Gaver, the problems that hit Ariane-1 are now solved — or as solved as anything can be in the space business.

After Ariane-3 will come Ariane-4, the first parts of the first flight model of which are to be completed this year. Ariane-4, to fly in March 1986, is designed around the next generation of communications satellites led by Intelsat VI: 4,200 kg and approaching 4 metres in diameter, comparable with the shuttle bay's 4.5 metres. The future of Arianespace depends on the company's success in gaining contracts for the Intelsat VI series, but knowing this, Intelsat can afford to wait. Around half the Intelsat business would be ample to give Arianespace a living, says Van Gaver.

The United States "made a mistake" in dropping conventional launchers in order to push business towards the shuttle, Van Gaver believes. Now the United States is trying to commercialize the old launchers

(Thor-Delta and Atlas) "but we have yet to see any contracts".

Ariane's other competitor is probably Japan — Van Gaver does not see the Soviet Union or China entering the Western telecommunications market. Japan's space programme is at present uncompetitive, but the Japanese space agency is developing a large cryogenic engine (such as Europe would only see on Ariane-5, now at the planning stage). "We must watch Japan carefully" Van Gaver says.

Robert Walgate

● Intelsat, the international communications satellite organization, has finally decided to go back to an old design for Intelsat V F8, due for launch on Ariane on 5 March. Problems with the L-band maritime communications system (see *Nature* 2 February, p.401) had pushed the launch back two months. Inmarsat, the organization which uses the L-band system, has complained that Intelsat V F7 is unworkable and has refused to accept it. Unable to solve the problem on F8, Intelsat has gone back to the F6 design, which still has problems but not so bad as those of F7. F9, which was to have been launched by Ariane this summer, has now been put back to 1985. □

US space station

Manufacturing plans launched

Washington

WITH a masterly sense of timing, the National Aeronautics and Space Administration (NASA) has announced that a major company, the Minnesota-based 3M Corporation, is planning to embark on a new programme of space research with the aim of eventually producing commercial products in orbit. The corporation's decision comes fast on the heels of President Reagan's announcement that the United States will build a manned space station within a decade, and is being portrayed by NASA as vindication of its claims that a space station could become the nucleus of a sizeable space-based manufacturing industry.

At a formal ceremony announcing 3M's plans, NASA administrator James Beggs did his utmost to link the company's decision with the President's approval of the space station. 3M, he pointed out, was the first non-aerospace corporation to sign a memorandum of understanding with NASA since the space station was approved. He said the deal would hasten the day when US industries were as comfortable with factories in space as they were with those on Earth.

NASA's enthusiasm is understandable. To persuade Congress to fall in with its plans for a space station, the agency must show that private industry (and foreign governments) are prepared to pick up a share of the tab. Propaganda value apart, the agreement with 3M looks decidedly one-sided. In return for expressing an

interest in space, 3M scientists will receive free access to NASA laboratories where materials processing research is under way. 3M also gets two free flights on the shuttle, the first as early as August.

3M's research programme will focus on processing organic materials in the low-gravity, high-vacuum environment available in space. Its vice president for technology services, Dr R. M. Adams, said the company's first experiment on board the shuttle would look at the growth of organic crystals and the development of thin films with novel physical and chemical properties.

Directed by both President Reagan and Congress to encourage commercial investment in space, NASA faces an uphill struggle in extending commercialization beyond its one great success story — the satellite communications industry which does more than \$2000 million of business a year. The most visible commercial interest in space-based manufacturing has come from Johnson & Johnson and McDonnell Douglas, which are developing a prototype instrument for the large-scale production of drugs by electrophoresis in space.

To garner more investment, NASA plans to offer an expensive array of incentives including free or subsidized flights on the shuttle and access to NASA expertise. That could queer NASA's pitch with academic scientists, many of whom have been grumbling for years about the length of the waiting list for space on the shuttle.

Peter David

High-energy physics

Future accelerator plan

Chicago, Illinois

AN enormously powerful colliding particle accelerator, the Superconducting Super Collider (SSC or the "desertron"), may still be only a gleam in the eye of US physicists but last week a workshop on the options for the proposed machine brought its construction a little nearer. SSC, at 20 TeV per beam, would be 20 times more powerful than even the Fermilab collider, which is still under construction.

The workshop, held at the University of Chicago and co-sponsored by Argonne National Laboratory, brought together about a hundred high-energy experimenters and theorists to sharpen the arguments on the relative merits of proton-proton colliders and antiproton-proton colliders. Either could still be the basis of SSC, which was recommended last year by the US high-energy physics community as the next big step in US accelerators.

Antiproton-proton colliders — such as the one at the European Organization for Nuclear Research (CERN) at Geneva and that being built at Fermilab — can circulate both types of particles within the same beam pipe and thus need only one ring of magnets. At 90 km in circumference, a one-ring SSC could cost considerably less than a two-ring proton-proton machine. However, antiprotons cannot be produced as copiously as protons. Antiproton colliders thus have lower luminosities and produce fewer particle interactions.

But the disadvantages of an antiproton collider may not be so great as had been thought. For example, the antiproton source group, headed by James Simpson of Argonne and Thomas Collins of Fermilab, concluded that antiproton sources can be built that would provide luminosities greater than 10^{32} per cm^2 per second. This approaches the 10^{33} that has generally been assumed possible in a proton-proton machine and necessary for much of the expected physics. In addition, most of the groups reported that luminosities much above 10^{32} would usually be unnecessary.

A proton collider can always be made more luminous than an antiproton machine, Collins told his audience. "But let's not choose a machine based on capabilities that we don't really need." However, the groups studying tests of electroweak interactions and potential new particles and interactions decided they could sometimes use all the luminosity they could get. Others, according to Robert Cahn of the Lawrence Berkeley Laboratory, really would like both types of collisions. But when the next major workshop on SSC takes place in June at a three-week meeting in Snowmass, Colorado, the participants will have two strong candidate accelerators to consider. **Larry Arbeliter**

UK pollution control

Commitment urged

THE Royal Commission on Environmental Pollution, in its 10th report, published this week, urges the British Government to "play a more positive role" in promoting environmental issues in the European Community. In a survey of its 14 years of offering advice on matters of pollution, the commission concludes that British commitment to international action "has not always been as strong as it might be". And on the vexed question of acid precipitation, concern over which is currently spurring several EEC draft directives on air pollution, the commission says that the Central Electricity Generating Board (CEGB) should within the next five years introduce pilot plants to investigate the various options for reducing emissions of sulphur dioxide from its power stations.

The EEC draft directives on air pollution controls, which are being pushed hard by West Germany, are currently the subject of an inquiry by a House of Lords Select Committee. This week the committee will hear a CEGB claim that one of the major proposed directives (on large combustion plants) misquotes figures from a cost-benefit analysis of sulphur emission reductions. The Confederation of British Industry and the Chemical Industries Association will join CEGB in condemning the draft directive as "excessive" and "very damaging". It calls for, among other things, a 60 per cent reduction in total national emissions of sulphur dioxide and fixed emission limits for sulphur dioxide from large plant. A separate draft directive on air quality limits for nitrogen oxides is also attacked for being too stringent.

The Royal Commission on Environmental Pollution has very widely drawn terms of reference and has never been afraid to use them. The commission, whose present chairman is Professor Sir Richard Southwood, Linacre Professor of Zoology at the University of Oxford, delivers a well-aimed swipe at Whitehall officials who have allowed ministers "unfettered discretion" over timetables to frustrate the wishes of Parliament. Implementation of the 1974 Control of Pollution Act has been piecemeal and incomplete, according to the commission, and "reflects little credit on successive administrations". Such action as has been taken is ascribed to pressure from the EEC. The lack so far of any government response to several of the commission's earlier reports is described as disappointing.

Official secrecy over pollution control has long been a controversial issue in Britain, and the latest report from the commission reaffirms its position firmly on the side of the "right to know" camp. No less than ten detailed recommendations urge greater public access to information compiled by pollution enforcement authorities. The commission says that "a guiding principle

for all legislative and environmental controls . . . should be a presumption in favour of unrestricted access" with provision for secrecy only in exceptional cases. Observing that secrecy fuels fear, the commission draws attention to public anxiety over nuclear power and calls for greater public representation on bodies concerned with managing nuclear waste.

Recognizing that there is a growing awareness of the urgency of international pollution problems, the Royal Commission advocates more forward planning of measures designed to avoid pollution before it becomes a problem. Thus it recommends government support for research into new potential pollutants from high-technology electronic and biotechnology processes, as well as more "traditional" pollutants in air and water. The government should also study how pollution control equipment is subsidized in other countries with a view to introducing subsidies in Britain. The commission says the politician faced with decisions on pollution control "cannot wait for rigorous proof"; prudent abatement practices must be instituted when perceived risk of damage justifies the cost of alleviation. It therefore proposes that decision makers should be guided by principles of the best practicable environmental option and the best practicable environmental timetable. The commission ends by lamenting the decreasing emphasis on environmental protection in Britain and the concomitant decrease in resources. **Tim Beardsley**

The Times 6 February

QUEEN MARY COLLEGE UNIVERSITY OF LONDON APPLIED MATHEMATICS DEPARTMENT

Theoretical Astrology Unit

Applications are invited for 1 or 2 year RESEARCH FELLOWSHIPS (with possibility of extension for 3rd year). The core staff of the Unit comprises 6 permanent members of the Academic Staff (Prof. Roxburgh, Drs MacCallum, Papadoulou, Rowan-Robinson, Schwartz, I. D. Williams) and 4 other staff.

The Times 15 February

Nothing has been said in the Theoretical Astrology Unit. Was this just a typographical error or some mysterious intervention that defies scientific explanation?

I suspect it is now too late to stem the flood of applications from would-be experts in the occult, but may I use your columns to point out to them that, had they been in possession of such skills, they would have known that their applications would be rejected: they should therefore not be surprised if they hear nothing further from us.

Yours etc,
IAN W. ROXBURGH,
Theoretical Astronomy Unit,
Dept of Applied Mathematics,
Queen Mary College

Acid rain

Canada must act alone

Washington

THE Canadian Government, exasperated by the Reagan Administration's decision to do no more than continue research on the acid rain problem, is considering substantial unilateral curbs on its sulphur dioxide emissions.

In 1982, Canada unilaterally decided to institute a 25 per cent reduction by 1990 in sulphur emissions. It has since been hoping to negotiate further cut-backs as part of an overall agreement with the United States in which both parties would work to reduce transboundary sulphur pollution. Although sulphur emissions from both countries cross into the other, Canada suffers most from transboundary pollution.

The Reagan Administration has consistently refused to order reductions in US sulphur emissions, arguing that scientific uncertainties must first be resolved. That position was reiterated most recently by science adviser George Keyworth in his briefing on Reagan's 1985 budget proposals, which include funds for more research on acid rain, but no regulatory action. The administration, in maintaining this position, has ignored the recommendations of both the National Academy of Sciences and a panel of scientific experts assembled by the White House Office of Science and Technology Policy. The White House group last summer found that although scientific uncertainties do exist, new regulatory action is justifiable because of the possibility of "irreversible damage" to the environment by acid deposition.

Canada's current programme involves reducing sulphur emissions 25 per cent below the levels already required to meet ambient air quality standards instituted in the mid-1970s. Canada's ambient standards are at least as strict as US standards and, like them, are designed to protect human health from acute effects of sulphur dioxide rather than to control acid rain.

Bruce Jutzi of the Canadian Embassy in Washington says that while Canada had not yet decided to institute new curbs, such action is under serious consideration. "Now that it's apparent there's nothing for the foreseeable future to be done together, that puts the onus back on the Canadian Government", he said. Jutzi said that ultimately Canada expects to have to reduce its sulphur emissions by 50 per cent from the ambient standards as its contribution to solving the transboundary pollution problem. "I'd personally be a bit surprised to see us go all the way to that limit without any movement from the United States", he said.

Canada's main source of sulphur emissions is smelters; in the United States it is coal-fired power stations.

Stephen Budiansky

Conservation

Farm policy hits Broads

CONSERVATIONISTS in Britain are anxiously awaiting the outcome of a local dispute that is seen as a major test of the 1981 Wildlife and Countryside Act. The question is who should pay the compensation that the act offers to landowners who agree not to turn valuable habitats into farmland. The growing incentives to produce more farmland were stressed last week at a meeting called to discuss the ecological crisis facing the locality.

The dispute concerns Halvergate Marshes, close to Great Yarmouth in eastern England, and has arisen because the Broads Authority, set up in 1978 to safeguard the whole of the Norfolk Broads of which Halvergate is a part, claims that it cannot afford the level of compensation involved. Two thousand acres of grazing marsh have been turned over to arable farming in the past 3 years and according to one estimate another five thousand acres could be ploughed within the next 5 years, unless compensatory payments of up to £200 per acre per year are found.

The problem of much of broadland is that it is very expensive to protect, having a high amenity value to tourists (whose boats are eroding the river banks) and to farmers (who drain and turn to the plough the prized grazing marshes in order to take advantage of the generous guaranteed prices laid down in Brussels). And so the area tends to throw into sharp relief conflicts of interest, and tests to the limit the legislation designed to resolve them.

The essence of the Wildlife and Countryside Act is that designated authorities wishing to protect a site from some planned action must, if persuasion is ineffective, be prepared to pay the owner for loss of projected profits. For arable farming these can be high, and the Broads Authority says it cannot afford to pay for these "management agreements". Half of the authority's income comes from the local councils of the region, while the other half is from central funds through the Countryside Commission. The authority, which com-

prises local authority representatives, has refused to continue making payments on these terms.

In areas designated as National Parks (which the Broads are not), 75 per cent of local compensation agreements are met from Government. But the Countryside Commission, itself the government's adviser, has argued strongly that 90 per cent reimbursement would be more appropriate for the Broads Authority. Representatives of the authority are soon to meet Mr William Waldegrave, Under-Secretary of State in the Department of the Environment, to thrash out a solution.

The powers of the Broads Authority have recently been the subject of a searching review by the Countryside Commission, which next month will formally recommend to government that it should be given special statutory powers to coordinate a rescue plan for the area.

The main problems facing broadland are that raised nitrate and phosphate levels cause algal blooms which shade out bottom-living plants. Consequently, banks erode more quickly, much wildlife disappears and sedimentation rates increase. Some ecologists fear that the expansion of arable farming may have wider consequences in the region.

Last week, two learned societies spent a day discussing the ecological crisis facing the Broads and heard speakers express concern that the European Community's Common Agricultural Policy and grants from the Ministry of Agriculture, Fisheries and Food will encourage the loss of much of the remaining grazing marsh in the area. At the end of the day, representatives of the British Ecological Society and the Linnean Society of London were told by their chairman that while it would be "entirely inappropriate" for them to pass a resolution expressing concern on the issue, it would be in order for a report on recent research "showing the scientific consensus" to be prepared and despatched to relevant government officials.

Tim Beardsley



Anathema to Europe's farmers — Halvergate Marshes

McNamara's plan

SIR — We suggest a strategy for global disarmament of nuclear weapons: that all land-based missiles carrying nuclear warheads be destroyed. We refer to a statement by Robert S. McNamara in *Newsweek* of 5 December 1983 and repeated at the meeting of the Bellerive Group in Geneva last December that nuclear weapons have no military use whatsoever other than to deter one's opponent from their use. With the words of Professor Robert Neild, Cambridge, at that same meeting: "we could draw back the nuclear weapons to where they belong, deep in the oceans, where they could serve the sole purpose of deterring nuclear attack or threat". We note that there seems to be general agreement that a limited nuclear exchange cannot be confined, but will lead to a general exchange of strategic weapons. If so, land-based missiles do not add to deterrence over and above what submarine-based missiles can achieve.

The nuclear arms deployed on land thus only reduce the security of the nations possessing them. The risk of nuclear war triggered by accident or misinterpretation (such as a computer error or an astrophysical phenomenon such as the 1908 Tunguska event misinterpreted as a nuclear attack) is greatly increased by the mere presence of stationary nuclear weapons on the continents which, being vulnerable, call for a fast response. Submarine-based nuclear missiles cannot be easily found or destroyed by enemy forces and thus do not invite overreaction in response to or anticipation of a presumed first strike.

The specific disarmament measure we suggest can be undertaken unilaterally without any loss of security. Any nuclear power implementing this strategy will gain a colossal political victory.

ANDERS BOSERUP

*Institute of Sociology,
DK-1361 Copenhagen K,
Denmark*

OVE NATHAN

*University of Copenhagen,
DK-1168 Copenhagen K,
Denmark*

TOR NOERRETRANDERS

*Science Department,
The Weekly Weekendavisen
DK-1147 Copenhagen K, Denmark*

Improper psychiatry

SIR — I was interested to read (*Nature* 304, 293; 1983) that "... remaining members (of the World Psychiatric Association) should put their houses in order. . .". You meant, I suppose, that neither unconditional nor automatic legitimacy should be granted to WPA when, as now, the bad boys are out. I cannot but agree with such a view. However, in the same article, you use the term "mental illness" when referring to several types of anomalous human behaviour. The nature and/or the very

existence of such "illness" remains a matter of debate, as you make clear, so I find it misleading when such "illness" is referred to later in the article. This shows how hard it is to avoid the use of an ambiguous term, even in a scientific journal.

This confusion could lead the reader to believe that scientifically unequivocal correlations have been found between truly existent mental illnesses (of a total physical origin), social danger (as an effect of such an illness) and treatments (scientifically based). Of course, if such correlations exist, they are far from being unequivocal and, beside mental "illness", social danger seems to be of a very doubtful consistency too. Even more doubtful is the therapeutic value of treatments based on physical modifications of the human brain (electroconvulsive therapy, lobotomy, heavy drugs) which, it is claimed, work simply as repressive tools. This is as close to torture as to therapy.

Put simply, the problem of ill use of psychiatry is psychiatry itself. The alleged use of psychiatry in repressing political dissent — an extreme degeneration of an already strongly criticized activity — provides an opportunity for pushing WPA and the United Nations to face their responsibilities. As for the Soviet Union's resignation from WPA, it should not be welcomed unconditionally, as it can be justified as a way of maintaining social prestige, credibility and income for the remaining members of WPA. Moreover, unpleasant though it is, we must face the possibility that such a resignation can be represented as a protest or a Soviet psychiatrist's refusal to play the role of scapegoat.

Finally, can we be sure that the type of abuse of psychiatry as documented by various international human rights organizations happens only in the Soviet Union? Should we draw the sad conclusion that what in the Soviet Union is done for political reasons is done elsewhere for ill conscience and/or for material benefit?

FERRUCCIO APRILE

*via Franco Bartoloni 40,
cap 00179 Roma, Italy*

Frost tolerance

SIR — Current ideas on the protection of plants from frost by alteration of the characteristics of the bacteria normally resident on the leaves raises an environmental question because of the differences between resistant and non-resistant plants, and the role of supercooling in each of these when they are exposed to frost.

The cultivated potato, some beans and the dahlia are examples of non-resistant plants which are damaged by ice forming within their tissues but not by sub-zero temperatures if no ice forms. This is possible if the water they contain supercools as the temperature passes below freezing point. In the great outdoors, the surfaces of leaves are normally quite dirty with all manner of material, some of which may be

expected to be of the sort which encourages water to freeze when the temperature falls below zero. The connections between events on the surface of leaves and the freezing of water within are unclear, but it is certainly true that the freezing of dew on the surface can lead quickly to ice formation within. It has been shown that some bacteria normally resident on leaves are able to encourage ice formation by virtue of some property of their coat. Genetic engineering has succeeded, it is claimed, in creating forms of these bacteria which lack the ability to trigger ice formation in water below its freezing point. It is also being suggested that if the natural population of bacteria on leaf surfaces of frost-susceptible crops could be replaced by these altered forms, then the plants would be less likely to freeze during frosts and so avoid damage.

Some cases of frost resistance by avoidance, apparently involving a thick water repellent cuticle, are known in natural vegetation. However, the majority of frost-resistant plants are quite different, they are tolerant of freezing dew and the formation of ice within the leaves and stems. Although like non-resistant plants their cells are killed if ice forms inside them, there are mechanisms which ensure that ice forms only between the cells and in less important tissues; the cells become desiccated due to water loss to the intercellular spaces, but few freeze. These mechanisms take time to work, and it is essential that in these plants the process of ice formation begins as soon as possible after the temperature falls below zero. Frost-tolerant vegetation is usually inoculated by freezing dew which sets off internal ice formation, and if this triggering fails, as it does when there is no dew because the air is too dry, then the subsequent "black" frost is more than usually damaging. The reason is that the plants have supercooled to a temperature well below zero, and when ice formation eventually begins it is a very rapid process. This is damaging because the movement of water out of the cells to the growing ice crystals cannot keep up with the rate of ice formation, and the ice grows into the cells and kills them.

Because of the different role of supercooling in the survival of these two main categories of plants, the expected results of any treatment which encourages supercooling will be different too. The question is, what may happen if such a treatment, carried out on a crop, also affects the surrounding natural vegetation? If the treatment works at all, the crop may be less damaged but the natural vegetation may be more damaged than would have been expected from the severity of that particular frost, unless the frost were so slight that both types of plant survive by supercooling.

D. B. IDLE

*Department of Plant Physiology,
University of Birmingham,
Birmingham B15 2TT, UK*

Polygraphic interrogation

David T. Lykken*

The polygraph ("lie detector") test is wrong one-third of the time overall, biased against innocent and conscientious persons, and can be "beaten" by sophisticated liars. Increasing use of this technique, in the United States and soon in Britain, is a cause for alarm.

THE origins of lie detection are lost in the mists of prehistory. When *Homo sapiens* developed language, one suspects that he soon began to use that language to mislead and deceive, mendacity following hard upon capacity. This in turn required all of us to become human lie detectors.

Although most of us can probably detect lying with better than chance success using just our eyes and ears and commonsense, some are less skilful than others. It is interesting that English, at least, is replete with terms descriptive of the low end of this dimension of talent ("gullible", "credulous", "sucker" and so on) yet apparently empty of descriptors for the skilled end ("suspicious" is biased, "perceptive" is too broad). The ancients developed rituals and ordeals for identifying liars, some of which, like modern lie detectors, relied on the autonomic arousal that accompanies fear. One often-cited trial required suspects to chew a mouthful of rice and then attempt to spit out a moist bolus. When the rice grains clung to the dry mucosa or dribbled individually from the parched and trembling lips, the priests believed that they had found their culprit¹.

In London in 1730, Daniel Defoe published a tract entitled *An Effectual Scheme for the Immediate Prevention of Street Robberies and Suppressing All Other Disorders of the Night*. Defoe argued that "there is a Tremor in the Blood of a Thief, that, if attended to, will effectually discover him". "Some are so hardened in Crime", he continued, "that they will . . . outface even a Pursuer; but take hold of his Wrist and feel his Pulse; there you will find his Guilt."

The first modern to take up Defoe's idea was also a writer of popular fiction, the inventor of the comic-strip character *Wonder Woman*. William Moulton Marston, a Harvard-educated psychologist and lawyer, realized, however, that fear symptoms alone are ambiguous for there may be a tremor in the blood of an innocent accused as well. Marston, who either coined the term "lie detector" or took up the phrase from one of the journalists whom he cultivated, believed that there is

an involuntary physiological response, like the elongation of Pinocchio's nose, that we show when, and only when, we lie. This specific lie response, Marston claimed, is a transitory elevation in systolic blood pressure brought about by the interaction of fear of detection and the attempt to maintain outward composure².

The polygraph

A Californian police officer, John Larson, soon developed the first recording polygraph which made continuous tracings of breathing movements and "average" blood pressure. On a moving paper chart, the "cardio" pen, connected pneumatically to a standard blood pressure cuff on the subject's arm, deflects briefly with each heart beat and the entire "cardio" tracing moves up or down with transitory changes in blood pressure. While it soon became obvious to Larson that Marston was mistaken, that innocent persons too may show blood pressure responses to accusatory questions, he nonetheless found his polygraphic lie detector to be a useful investigative tool. When questioning a series of possible suspects in some crime, it often happened that one of them would display a really strong reaction to the relevant or accusatory question and, moreover, when confronted with this evidence of internal disquiet, such suspects would frequently confess.

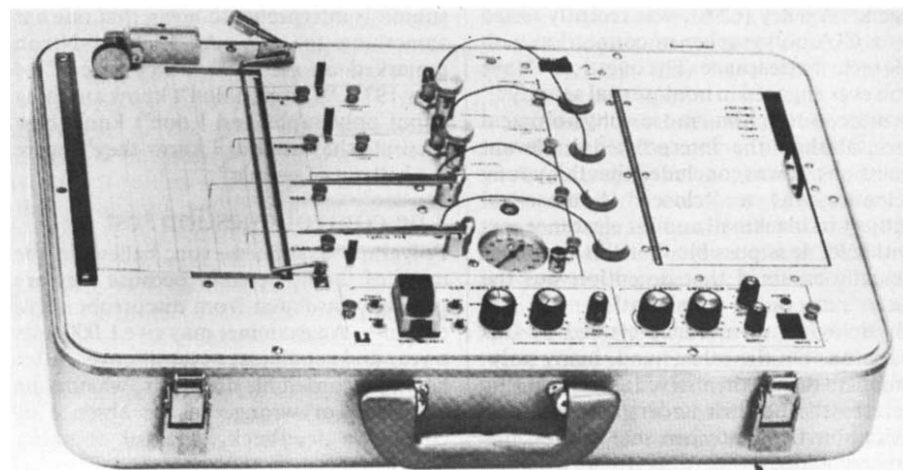
Like the rest of us, police have always looked for blushing, trembling, averted eyes and other signs of guilty nervousness

when questioning someone whose testimony is in doubt. Here was a scientific instrument that could record internal blushes and other "tremors in the blood" and which seemed to be effective. The standard modern polygraph is not much different from Larson's. Two pens are driven by pneumatic tubes placed about the subject's chest and stomach, recording thoracic and abdominal breathing movements. The "cardio" pen is still attached to a blood pressure cuff which, during the test, is inflated midway between systolic and diastolic pressure, partially occluding blood flow to the arm. The fourth pen is connected electrically to a pair of metal electrodes on the subject's fingers: it records changes in the electrical resistance of the skin, electrodermal responses which reflect sweating of the palms.

A single polygraph chart is a continuous record of these variables over the 4 or 5 minutes required to ask a series of about 10 questions; at the end of each chart it is necessary to release the cuff pressure for a time to restore circulation in the arm. Most polygraph tests involve three charts, three repetitions of the same list of questions.

The relevant/irrelevant test

Leonarde Keeler, Larson's assistant, developed a portable polygraph instrument and, in 1930, moved to Chicago where he established the Keeler Polygraph Institute, the first school devoted to the training of polygraph examiners. There are now dozens of such schools in the United States,



A typical four-channel field polygraph.

*Department of Psychiatry, University of Minnesota, Minneapolis, Minnesota 55455, USA.

more than 20 in Florida alone, most of which offer a curriculum lasting from six to eight weeks or about one-sixth of the study time required by the average barber college.

The standard Keeler polygraph test involved a series of relevant or "Did you do it?" questions ("On 15 May, did you take the \$1,000 from the safe?") intermixed with a number of innocuous irrelevant questions ("Is today Tuesday?"). Since the relevant questions can span a lot of territory ("Did you tell the truth on your application form?"; "Have you been in contact with a foreign intelligence agency?"), this is the test format commonly used in screening job applicants or vetting people for security clearances. It is estimated that a million people in the United States were subjected to polygraph testing in 1983. President Ronald Reagan's National Security Directive, due to take effect in April 1984, will require millions of federal employees and employees of civilian defence contractors to submit to such tests on demand or face "adverse consequences".

All anyone can determine from a polygraph chart is that the subject was more aroused or disturbed by one question than by another; one cannot tell why he was disturbed, or whether the question elicited guilt or fear or anger. Nor is it meaningful to compare Jones's reaction with Smith's response to the same question, since individual differences both in emotional and physiological lability are so great that a small response for one person may be a large response for another. Moreover, it is generally agreed that it is the question, rather than the subject's answer to the question, that determines the recorded response. In the relevant/irrelevant test, therefore, it must be assumed (1) that an innocent person will be no more disturbed by the accusatory relevant questions than by the innocuous irrelevant questions and (2), the corollary, that any relatively stronger response to a relevant question indicates deception rather than, say, embarrassment, fear or indignation.

A young engineering professor, engaged in secret research for the Central Intelligence Agency (CIA), was recently tested by a CIA polygrapher in connection with his security clearance. The question "Have you ever engaged in homosexual activity?" produced in him more physiological arousal than the interpolated irrelevant questions. It was concluded that this young scientist was a "closet" homosexual subject to blackmail and his clearance was withheld. It is possible that this examiner actually assumed that deception was the only reasonable explanation for the physiological disturbance evoked by this question. On the other hand, many polygraphers regard themselves as the real "lie detectors" and their understanding of the facts plus their interpersonal impressions are combined, according to unspecified rules, with whatever they think they can see

Polygrapher's diagnosis

Status of suspect	Deceptive	Truthful	Total	Accuracy
Guilty	98.1	19.9	118	83%
Innocent	38.2	50.8	89	57%
Accuracy*	72%	72%	20%	72%

Aggregated findings of the three credible field studies of polygraph accuracy^{6,8,9} in which polygraph charts of 207 criminal suspects were scored independently by 14 polygraphers. Average accuracy on guilty suspects was 83%; however, 47% of innocent suspects were erroneously diagnosed as "deceptive".

*The accuracy of tests that are "failed" or "passed" depends on the proportion of liars in the group tested. Thus, if 800 of 1,000 persons tested are truthful, a test that is 72 per cent accurate overall will "fail" 144 liars and 224 truthful persons.

in the charts in arriving at their diagnosis. Moreover, many polygraphers continue to believe, against all evidence, that certain patterns of reaction are specific evidence of lying; "stair-step" respiration, for example, or an increase in blood pressure in anticipation of a question, followed by a "relief response" after the question has been answered. A polygraph chart is noisy and complex; like the tea leaves at the bottom of a cup, it invites flights of interpretive fancy.

Polygraphic interrogation

Reacting to President Reagan's plans vastly to expand federal use of the polygraph test, the US Congress asked its Office of Technology Assessment (OTA) to examine the scientific status of this technique. OTA's report³, published in November 1983, concluded that there is no scientific evidence at all concerning the validity of the type of test used in pre-employment or periodic screening. To understand how, in the absence of such evidence, polygraphic screening has become a multi-million dollar industry in the United States, it is necessary to appreciate that the lie detector has been a part of American mythology for 50 years. Every citizen has heard of this device and assumes that it must work; why else would the Federal Bureau of Investigation (FBI) and the Pentagon use it?

Why would politicians and other celebrities, their veracity challenged, volunteer to take lie detector tests? Once established, such a myth feeds on itself. Refusal to submit is interpreted to mean that one has something to hide. As Richard Nixon remarked on the Oval Office tape of 14 July 1971, "Listen, I don't know anything about polygraphs and I don't know how accurate they are but I know they'll scare the hell out of people".

The control question test

Polygraph examiners, too, believe in the myth of the lie detector because they are generally insulated from corroborative evidence. An examiner may give 1,000 tests a year and yet almost never discover, after having recorded his diagnosis, whether he was right or wrong. In the absence of corrective feedback, wishful thinking flourishes.

Yet, by the late 1940s, some perceptive

polygraphers had come to realize that, "Did you take the \$1,000?" may be a stronger stimulus than "Is your name John?" even for an innocent suspect. Therefore, a new test format was devised by the late John Reid, an attorney, who operated the Reid College for the Detection of Deception in Chicago. Reid added a third type of question, misleadingly known as "control" questions, to the standard list. Although it does not lend itself to the screening application, the control question test (CQT), in one form or another, has become the standard technique for use in specific issue situations, as in criminal investigation.

For a person suspected of Crime X, a genuine control question might be, "Did you commit Crime Y?", where Y and X are equally serious and where the examinee can be persuaded to believe that he is truly suspected of Crime Y although, in fact, the examiner knows that Crime Y is fictitious. Then the polygraphic reaction to the question about Crime Y will provide an estimate of how this subject should react to the question about Crime X, if he is also innocent of that crime. For a variety of obvious reasons, this type of control is seldom possible. Instead, the examiner tries to develop two or three "probable-lie control questions", questions referring to the examinee's past which he will answer deceptively out of embarrassment or a desire to make a good impression.

This curious concept can most easily be understood in terms of an example. A young mother in Hillsville, Virginia, was awakened at 3 a.m. one night last year by an intruder who, on threat of death to her and her child, proceeded with an elaborate sexual assault which, among other perversities, involved shaving her pubic hair. The local sheriff had read somewhere that this feature was characteristic of lesbian rape and the victim therefore was asked if her assailant had been a woman. In many US jurisdictions it is commonplace to require rape victims to submit to a polygraph test and, when this woman insisted that she had been raped by a man, she was referred for testing. Two of the "control" questions asked were: "Before the age of 15, do you remember playing with your sex organ?"; and "Between the ages of 15 and 25, did you ever have sex with a woman?" The examiner, employed by the state crime

bureau, had been trained at the highly regarded Backster school of polygraphy and had more than 10 years of experience. On the basis of that training and experience, he assumed that this woman's "No" answers to these questions were "probable lies". Therefore, he compared the strength of her physiological reaction to these "control" questions with her reaction to the relevant question, "Was the person who attacked you a woman?" Backster, whose methods are used by FBI and taught at the US Army Polygraph School, teaches that a subject will focus on the source of greatest threat. If he can be truthful in answering the relevant questions, then the examinee is expected to feel that his greatest jeopardy lies in the "fact" that his answers to the control questions are deceptive.

Since the Backster form of CQT is widely regarded as the state of the art of polygraph interrogation, it is appropriate to consider its assumptions with some care:

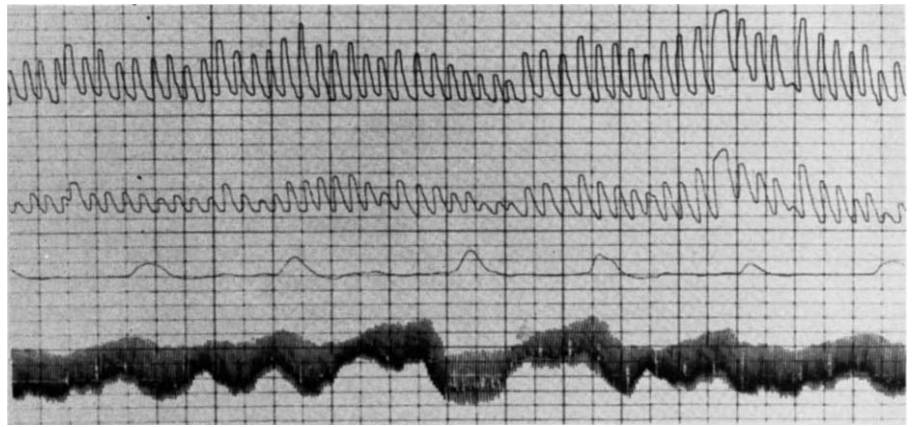
Assumption 1

The examiner will be able to invent several questions referring to the subject's past which the subject will answer deceptively. Is it reasonable to assume, in spite of her denials, that this rape victim practised masturbation before the age of 15 and that she subsequently had at least one homosexual experience? In 1982, in connection with a leak of information to the *Washington Post*, the chairman of the Joint Chiefs of Staff and the Secretary of the Army were given polygraph tests by Pentagon examiners. The questions used in those tests are not in the public domain, yet we can still ask ourselves whether it is reasonable to assume that those examiners were able to invent questions to which these high officials "probably lied".

Assumption 2

A subject who can answer the relevant question truthfully will be more disturbed by the "controls" than by the relevant questions. In the case of this rape victim, perhaps all one can say is that it is unreasonable to predict with any confidence which questions she might find more disturbing.

Floyd Fay, on the other hand, was confronted with a clearer choice⁴. Fay was arrested in Toledo in 1978 and charged with robbing and murdering an acquaintance who, before dying, had stated that the masked robber "looked like Buzz (Fay)". Fay was held without bond for two months while the police searched, in vain, for evidence tying him to the murder. Finally, the prosecutor offered to drop the charges if Fay passed a polygraph test, but required Fay to stipulate that the results should be admissible in court if the test indicated deception. Fay agreed, failed the test, failed a second test by a different examiner, was tried and convicted of aggravated murder and sentenced to life imprisonment. After more than two years, the real



A section of polygraph chart. The top two channels record thoracic and abdominal breathing movements. The third pen records changes in the electrical conductance of the skin; the wave-like electrodermal responses are related to insensible sweating of the palms. The bottom trace is the "cardio" channel; the pen deflects with each heart beat and the entire tracing moves up and down with changes in blood pressure.

killers were caught; they confessed, exonerating Fay who was promptly released.

A relevant question in Fay's first test was "Did you shoot Fred?" A "control" question was, "Before the age of 25, did you ever think of hurting someone for revenge?" Because Fay's physiological response to the first question was, in the examiner's opinion, somewhat stronger than his reaction to the second, Fay was diagnosed as "deceptive". What the jury heard was that this examiner had many years of experience, that he had administered a standard control question polygraph test and that in his professional opinion Fay was deceptive in denying his guilt. Such is the strength of the myth of the lie detector that the jury had little difficulty in concluding "beyond reasonable doubt" that this man, who had no previous criminal record and against whom there was no credible evidence, was guilty of murder.

The two tests that Fay "failed" were typical examples of the CQT format which spokesmen for the polygraph industry claim to be accurate at least 95 per cent of the time. F. Lee Bailey, for example, a polygrapher before he became a defence lawyer, asserted recently on national television that, "Out of 100 tests, we expect 96 to be correct, three to be inconclusive and only one to be in error". To accept such a claim, we must be able to agree that an innocent person, accused of killing Fred, will be less disturbed by the question, "Did you shoot Fred?" than by the question, "Before the age of 25, did you ever think of hurting someone for revenge?" — in 96 cases out of 100.

Assumption 3

The third assumption of CQT is that guilty persons, who must answer the relevant questions deceptively, will show stronger reactions on the polygraph to the relevant than to the "control" questions. While this seems plausible, at least in comparison with the first two assumptions, it should not be accepted too readily. John Reid himself showed in 1947 that a sophisticated subject can artificially augment his natural

response to a question by self-stimulation — by biting his tongue or pressing on a nail hidden in his shoe — and that the examiner cannot distinguish these augmented responses from normal ones. Thus, a person who can recognize the "control" questions as they are asked might "beat" the lie detector by augmenting his responses to these questions so that they are stronger than his natural responses to the relevant questions. Floyd Fay confirmed this idea while serving time in the London (Ohio) Correctional Facility. That prison routinely used polygraph tests to adjudicate charges against inmates of violating prison regulations. Having studied the literature on the polygraph, Fay decided to attempt his own experiment. By the time he was exonerated and released, Fay had managed to coach 27 inmates, all scheduled to take polygraph tests and all willing to confess to him that they were guilty; 23 of the 27 beat the lie detector.

The evidence

The recent OTA report, which found no credible scientific evidence of the validity of R/I or screening tests, did find 10 field studies of the accuracy of CQT that it considered to be worthy of consideration. The earliest of these⁵ compared results of tests administered to criminal suspects with the judgement of a panel of legal experts who later reviewed the dossier on each suspect. Because the polygraphers in this study based their conclusions on what they knew of the evidence and on their impressions of the suspects' demeanour during the interview, as well as on whatever they inferred from the charts, we cannot tell what contribution, if any, the actual polygraph results made to their performance. To assess the accuracy of the lie detector in real life, we must use tests given in real life situations (since we cannot simulate in the laboratory the emotional concerns of an actual criminal suspect) and we must then have the polygraph charts evaluated by a different examiner from the one who administered the test.

Another requirement is to find a criterion by which to identify those

suspects who were actually lying and those who were being truthful. Some individuals will confess their guilt at a later time so that we know they were deceptive on the polygraph. Such confessions often will clear another suspect whose tests are then verified as truthful. Finally, it is plainly improper for a polygrapher to select the tests to be re-scored, choosing only those examples that he did score or would have scored correctly. Several of the other studies considered by OTA appear to have violated this rule.

In one good study⁶, for example, 109 successive suspects were tested by one examiner and then the charts were scored by a second polygrapher. A panel of five experts reviewed the final evidentiary files and excluded cases where they felt that the evidence did not permit a decision as to guilt. On the 51 cases where there was both a panel decision and a definite conclusion by the examiner, the average accuracy achieved was 72 per cent (against a 50 per cent chance expectancy). The polygrapher correctly labelled 98 per cent of the guilty suspects as deceptive, but he did this at the expense of the innocent suspects, 55 per cent of whom were also called deceptive. This study is one of the three extant that clearly meet the criteria specified above.

Subsequently, however, the guilt or innocence of 16 of the original 109 suspects was verified by confession and the polygrapher was shown to have been correct in 14 of these cases; he classified the other two as inconclusive. He then submitted these 16 charts to 25 other polygraphers for re-scoring, finding that those who used the same scoring system as he did scored these particular charts as he had done. This small study⁷ provides evidence that polygraphers who use numerical scoring will tend to agree with one another, but it cannot be taken as evidence of the accuracy of CQT. The original study, from which these few charts were drawn, provides that evidence and it shows that CQT was wrong 28 per cent of the time overall and that it was biased against the truthful persons, more than half of whom were erroneously scored as deceptive.

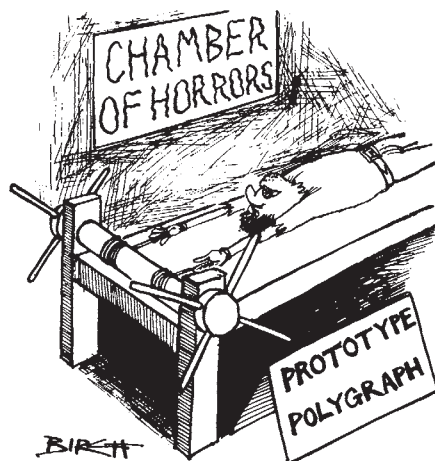
Guilty knowledge test

A fundamentally different procedure for the polygraphic interrogation of criminal suspects attempts to detect not lying but the presence of guilty knowledge. In the rape case described earlier, the victim's twisted assailant did a variety of unusual things during the two hours or so that he spent with her. When she chanced to identify him on the street four months later, the memory of most of those acts would still have been with him. The police examiner might have conducted a polygraph test using questions like:

"The rapist in this case poured some sort of fluid between his victim's legs. If you are the man, you will remember what liquid it was. Just sit quietly now and repeat the

names of these liquids. What liquid did the attacker pour between the victim's legs? Was it — (1) water? (2) beer? (3) nail polish? (4) urine? (5) perfume? (6) blood?"

Since people tend to respond most strongly to the first item in a series, the first alternative is never correct and never scored. An innocent person might react physiologically to each of the five scored alternatives but would have only about one chance in five of reacting most strongly to the correct alternative (nail polish, in this case). With 10 multiple-choice items of this type, an innocent person would have less than one chance in 10 million of reacting most strongly to all 10 correct alternatives in the absence of guilty knowledge. The guilty knowledge test (GKT), in laboratory experiments with simulated crimes, has repeatedly been found to detect most of the "guilty" suspects while clearing all of the innocent ones. In the absence of research on GKT under real life conditions, however, it would be rash to assume that it



can work equally well in actual criminal investigation. This research has not been attempted, partly because GKT is more difficult to implement than merely asking the suspect, "Did you do it?" and partly because polygraphers continue to believe in the myth of the lie detector. In the actual rape case, the same examiner who had concluded from testing the victim that her assailant was a woman also tested the man she later identified as the rapist. Not too surprisingly, this man was diagnosed as truthful.

Conclusions

The type of polygraph test used for screening job applicants (or federal employees requiring top secret security clearance) assumes that physiological reaction to accusatory questions is evidence of deception. There is no credible scientific evidence concerning the validity of such screening tests. Since highly socialized persons (religious, conscientious persons) tend to fail polygraph tests even when truthful, whereas poorly socialized persons — or sophisticated ones such as the

KGB agents trained at their anti-polygraph school in Poland — tend to pass such tests even though lying¹⁰, it is possible that the use of polygraph screening may produce results that actually are opposite to those intended.

The type of test used in criminal investigation or other specific issue situations also is predicated on implausible, even fantastic, assumptions. The three credible studies we have of the accuracy of these tests indicate that they are wrong nearly one-third of the time overall. The three studies show that an innocent person has almost a 50:50 chance of "failing" the lie detector. For this reason, when several persons are tested in respect to the same issue, polygraphers typically withhold judgement until all results are in. In the Pentagon case mentioned above, all 36 members of the Defense Resources Board were tested before one of them was identified as the culprit who leaked the information. Had the tests been scored independently, it is likely that a dozen or more "culprits" would have been identified. (The man who failed the polygraph, it turned out, was innocent; the real culprit apparently beat the lie detector. Even so, polygraphers here can claim 34/36 or 94 per cent accuracy!)

In the wake of the Geoffrey Prime spy case, and on the recommendation of the US security agencies, the British Government has decided to begin using the polygraph in vetting personnel of GCHQ and the Secret Service. Apart from damage done to the careers and reputations of innocent persons, this decision is likely to result in the loss to the government of some of its most conscientious civil servants, replacing them with the undersocialized types who easily pass polygraph tests. Because of the tendency to slightly more expensive but more effective security procedures, once polygraph testing has been introduced, this decision may well open the door to easy penetration of the security services by foreign agents trained to beat the polygraph.

The ever-expanding dependency on this pseudoscientific technique, in the face of both reason and evidence, implies an alarming degree of irrationality among decision makers in private industry, in the criminal justice system and even at the highest levels of government. □

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Abundant phase transitions

The bewildering variety in which phase transitions come is neatly illustrated in a collection of papers to be found in a single issue of a journal.

LAST year's final issue (on 26 December 1983) of *Physical Review Letters*, a journal which is no longer the exclusive preserve of high-energy physicists, contains half a dozen new twists in the understanding of phase transitions. What follows is merely a selection.

Simplest, perhaps because it is experimental, is a study of the properties of plutonium antimonide (chemical formula PuSb) which, among other things, has entailed the preparation of what are said to be the first "sizeable crystals" of any plutonium compounds — they come out at 8 mm³. That in itself has been a substantial undertaking. Crystals of PuSb have been grown and embedded in a matrix of metallic copper (B.R. Cooper *et al.* *Phys. Rev. Lett.* **51**, 2418) by a four-cornered collaboration involving the Los Alamos National Laboratory, the University of West Virginia, ETH at Zurich and the Transuranium Institute at Karlsruhe (on account of its "unique handling and glove-box facilities"). The trick of surrounding the crystals with solid copper has the advantages of eliminating the toxicity hazard of the plutonium but also of preventing self-heating due to spontaneous fission.

The interest of PuSb is its magnetism. Plutonium is an element whose 5f shell is only partly filled with electrons. Indeed, the triply charged plutonium ions in the antimonide can have only five 5f electrons, whence the argument that the ion must have much in common with the electronic structure of the trivalent cerium ion Ce³⁺, which has only one electron in the 4f shell.

This expectation seems to be borne out by experiment. PuSb, a cubic crystal, has markedly anisotropic properties which imply that the f electrons are not strictly localized on the atoms which contribute them to the lattice but, rather, are stretched out towards their nearest neighbours. Cooper *et al.* argue that this is an interesting case of hybridization between atomic and band electrons. Their discussion of the strength of the magnetic coupling between neighbouring plutonium atoms in the lattice is necessarily incomplete, but does not obscure the striking circumstance that PuSb is a ferromagnetic material below 75K and an antiferromagnetic material above, with a transition to disorder at 85K.

The obvious interest of the data now published is that they offer a way of testing different methods of approximation to interionic forces — and that there are more

where these have come from. The antiferromagnetic phase of PuSb appears from neutron diffraction to have long-range order in which magnetic spins cancel out over a distance of about two lattice units.

This is comparable with the nature of the long-range order with respect to translation that occurs in the nematic phase of liquid crystals, where planar (and usually polar) molecules are spatially ordered. In the same issue of *Phys. Rev. Lett.* (p.2386), G. Grinstein and John Toner from the IBM Research Center at Yorktown Heights, New York, claim a remarkable prediction of a previously unrecognized property of those liquid crystals which can exist in the nematic phase and the two smectic phases *a* and *c*, the second of which involves both orientational and translation ordering of the planar molecules.

Which of the three phases will be assumed by any material will be a function of the only two adjustable parameters available — the temperature and the concentration of the material in its solvent. On such a plot, the pairwise phase boundaries should come together at a triple point, but so far this occurrence seems to have been unexplored. Grinstein and Toner argue that there cannot be a simple triple point but, rather, that there must be a fourth phase, a kind of hybrid between the nematic and smectic *c*.

Physically, what this implies is that the geometrical rearrangement of the planar molecules needed to accomplish the phase transition requires more than two steps to be taken in succession, but the point is established by means of A. C. Wilson's correspondence between lattice statistics and the renormalization theories that usually turn up in gauge field theories. None of this, of course, implies that the fourth phase will exist over a wide range of concentration or of temperature — indeed, the predicted effect may turn out to be too fleeting to be recognized.

The solidification of xenon adsorbed in gaseous form on a platinum surface has, however, been neatly observed by the scattering of helium atoms. Bene Prelsema, Laurens K. Verheij and George Comsa of the Low-Temperature and Vacuum Physics Division of the West German nuclear research centre at Julich point out (p.2410) that the cross-section for scattering helium atoms from molecules (for example CO) adsorbed on a metal surface can be very much larger than simple geometry suggests, perhaps as much as 125 Å². The

result is that it is possible to use helium scattering as a measure of aggregation of adsorbed molecules for each possible density of coverage. Hitherto, attempts to measure condensation in adsorbed xenon have been unsuccessful, chiefly because of the need to work with surfaces which have been densely covered with adsorbed molecules. The helium atom technique works, but at the opposite end of the range, with as few as one in a thousand adsorption sites occupied. The result is what must have been hoped for. The transition from two-dimensional gas to two-dimensional solid occurs at lower coverage by adsorbed gas with decreasing temperature (at least down to that of liquid nitrogen). It has even been possible to infer the heat of vaporization of solid two-dimensional xenon (1.1 kcal per mole) and, because the densities of adsorbed gas at which crystallization occurs are indeed low, to explain why the phenomenon has not been previously observed. While xenon itself is of moderate interest, the helium scattering technique is likely to be widely used.

No fewer than three other types of phase transitions crop up in the same issue of *Phys. Rev. Lett.*, of which the transition known as commensurate-incommensurate is perhaps the simplest. A. H. Moudden, D. E. Moncton and J. D. Axe from the Brookhaven National Laboratory describe (p.2390) measurements of the behaviour of the dipolar molecular thiourea in strong electric fields using X rays to look for periodicity in a crystal lattice. At high field strength, greater than 1,000 volts per mm, thiourea crystals behave as if successful slab-like domains in which dipoles are oriented in one direction or the opposite in an alternating fashion along the field. The numbers suggest that under these conditions the slabs may be either 7 or 8 molecular planes thick. Decreasing the electric field allows 9-fold slabs to occur and also suggests how the relaxation to low field conditions may show hysteresis, as is observed.

What else? The process of wetting a solid surface is also a candidate for phase transition while the problem of percolation, in which free moving molecules thread their way through a tortuous lattice, clogging the system if there are too many of them, is dealt with for the first time on a lattice of fractal dimensions, no doubt simulating more accurately what may happen in the percolation of petroleum through, say, a reservoir rock.

John Maddox

Genetics

DNA supercoiling and gene expression

from L. Mark Fisher

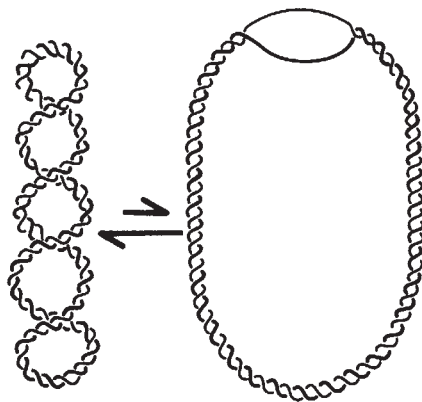
SUPERCOILING of DNA — coiling of the DNA helix axis — is now known to have a dramatic effect upon DNA replication, recombination and transcription in bacteria¹⁻³. But how are bacteria able to regulate the supercoiling of their DNA? Recent work reveals that the degree of DNA supercoiling depends upon a dynamic balance between two mutually antagonistic enzyme activities: DNA gyrase which supercoils DNA, and DNA topoisomerase I and perhaps other enzymes which remove supercoils. Intriguingly, expression of the genes for one of these enzymes — DNA gyrase — appears to be itself regulated by DNA supercoiling^{4,5}.

Circular chromosomal and extrachromosomal DNA is actively maintained in a negatively supertwisted state by the ATP-dependent bacterial enzyme DNA gyrase. When gyrase activity is blocked by chemical or genetic means, relaxation of cellular DNA rapidly ensues with pleiotropic biological effects, including the halting of replication^{2,3}. The coexistence in *Escherichia coli* of both gyrase and DNA-relaxing activity, predominantly DNA topoisomerase I (omega protein), suggests that both these competing activities may play a part in determining the cellular level of DNA supercoiling. Evidence to support this proposal has come from a series of elegant experiments made possible by the localization of the structural gene for topoisomerase I (*topA*) and the isolation of *E. coli* strains carrying mutations in this gene^{6,7}.

TopA maps to a region of the chromosome near *cysB* and is not closely linked to either *gyrA* or *gyrB*, which encode the gyrase A and B subunits. Point mutations in the *topA* gene of *E. coli* and their effects on DNA supercoiling have recently been studied in some detail^{8,9}. When a strain bearing the nonsense mutation *top10* was examined, a level of DNA supercoiling higher than that of the isogenic wild-type strain was found. As the *top10* strain produces only low levels of topoisomerase I activity the simplest conclusion is that topoisomerase I acts in removing DNA supercoils *in vivo*. Deleting the entire *topA* gene leads, however, to an apparently contradictory result — the degree of DNA supercoiling is lower than that of the isogenic *TopA*⁺ counterpart⁹.

This paradox was resolved with the discovery that deletion of the *E. coli topA* gene is accompanied by the acquisition of a secondary mutation, closely linked to *gyrA* in one strain and to *gyrB* in another strain^{8,9}. The respective gyrase A and B

subunits purified from these strains are partly defective and are less efficient in promoting DNA supercoiling than wild-type subunits⁴. Apparently, too much supercoiling caused by a total absence of topoisomerase I is as deleterious as too little. Compensation for a lack of topoisomerase I activity is achieved by spontaneous mutations that reduce the supercoiling activity of gyrase. Thus, selective



Underwound circular duplex DNA (right) forms negative supercoils (left). DNA in the form of a closed circle with about one helical turn for every ten base pairs — the same pitch as linear duplex DNA in the B configuration — is said to be 'relaxed'. If the two strands of the duplex circle are intertwined fewer (or more) times than in the relaxed circle, the DNA minimizes its departure from the energetically favoured configuration by coiling about its axis, as in the figure. By convention, the supercoils formed by underwound DNA are referred to as 'negative' and those by overwound DNA as 'positive' (see *News and Views* 294, 607; 1981).

pressure exists to maintain an intermediate biologically acceptable degree of supercoiling.

How is the degree of supertwisting regulated in wild-type cells? Experiments by Gellert, Menzel and co-workers demonstrate that synthesis of gyrase is itself controlled by DNA supercoiling^{4,5}. First, they carried out two experiments which showed that gyrase synthesis is activated by conditions that lower gyrase activity. Using antibodies raised against purified gyrase A and gyrase B proteins they measured the relative rates of synthesis of these proteins in various conditions. Treatment of *E. coli* with either of the gyrase inhibitors novobiocin or coumermycin, at concentrations known to induce relaxation of chromosomal DNA, led to a 10-fold increase in the rate of gyrase A and gyrase B synthesis. The time course of gyrase induction closely follows that observed for chromosomal DNA relaxation promoted

by the inhibitor. Next, a strain carrying two mutations in *gyrB*, one conferring temperature sensitivity to growth, the other coumermycin resistance, were shown to have normal levels of gyrase A and B proteins when challenged with coumermycin. A shift to the non-permissive temperature, however, resulted in increased synthesis of both gyrase A and B subunits when compared with the isogenic *gyrB*⁺ strain.

To confirm that DNA relaxation is the cause of gyrase induction, cloned *gyrA* and *gyrB* genes contained in plasmids were used to programme a cell-free protein synthesis system. The *gyrB* plasmid maintained in a relaxed state by the inclusion of novobiocin (to inhibit endogenous gyrase in the extract) produced up to 100 times more gyrase B protein than the plasmid in its supercoiled conformation. Stimulation of gyrase B synthesis was also observed in the absence of gyrase inhibitors when purified topoisomerase I from *E. coli* or HeLa cells was added to the system. Similar effects were seen in experiments using the *gyrA*-containing plasmid. These results show that gyrase synthesis is promoted by a relaxed DNA template and render unlikely the formal possibility that gyrase might regulate its own synthesis by binding to putative repressor sequences on DNA.

It is interesting that both the *E. coli* and the HeLa topoisomerase I enzymes act as positive effectors of gyrase synthesis. The fact that a eukaryotic relaxing enzyme can stimulate gyrase synthesis argues that these enzymes exert their effects by relaxing the DNA template rather than by binding to regulatory sequences on DNA. What controls the expression of topoisomerase I is not yet known although the recent cloning and analysis of the *topA* gene by Wang and colleagues should allow progress to be quickly made¹⁰. As yet, the possibility remains open that other DNA-relaxing enzymes isolated from *E. coli*, for instance topoisomerase II¹¹ (refs 11, 12), or topoisomerase III¹³, may operate in concert with gyrase and topoisomerase I.

GyrA and *gyrB* belong to an emerging class of genes whose expression is dependent on DNA conformation¹⁴.

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Promoters that are activated by DNA supercoiling have already been identified and include those for catabolite-sensitive genes — the maltose operons, the lactose and galactose operons and the tryptophanase gene¹⁵. The mechanism by which DNA supercoiling enhances transcription from these promoters is not well understood. What is clear, however, is that RNA polymerase unwinds DNA on binding to the promoter — a process which should occur more easily on negatively supercoiled DNA as it is already partially unwound¹⁶. Why then is gyrase synthesis activated by a

relaxed rather than a negatively supercoiled DNA template? At present this problem is unresolved although preliminary evidence again suggests that control is exercised at the level of RNA transcription¹. Structural and functional analysis of gyrase genes and their promoters should help clarify how DNA supertwisting differentially affects gene expression. □

L. Mark Fisher is in the Department of Biochemistry, St George's Hospital Medical School, University of London, Cranmer Terrace, London SW17 0RE.

Science journals

Cryptozoology

from Robert M. May

OF the 79 *News and Views* pieces I have written over the past ten years, the one attracting far and away the most attention outside the ranks of *Nature* readers was a brief *jeu d'esprit* on 'The ecology of dragons' (*Nature* 264, 16; 1976). This article was picked up by United Press International, and resulted in telephone calls from radio stations and newspapers in most English-speaking countries, offers to appear in three television shows and an invitation to write a coffee-table book about dragons. Needless to say, none of my other *News and Views* articles has produced anything approaching this reaction. The dragon article differs from the others only in that it deals with the unreal and fanciful, rather than with the real wonders that actually exist in the natural world. To generalize recklessly and gloomily, I think this isolated experience reflects a tendency on the part of the media — and maybe the public — to prefer meretricious marvels to real ones.

Be all this as it may, it is consistent with the fact that, of the plethora of new journals established in 1982–1983 (see the 6–13 October 1983 issue of *Nature*), *Cryptozoology* was the only one reviewed in *Newsweek*. *Cryptozoology* is the official journal of the International Society of Cryptozoology, whose president, Bernard Heuvelmans, defines the subject as "the science of 'hidden' animals". The first and to date only volume of the journal runs to 100 pages embracing articles, research reports, field reports and book reviews. By a rough count, 29 pages deal with 'wildmen' of one kind or another (such as 'Sasquatch' or 'bigfoot' in the Pacific Northwest and 'yeti' in China), 7 pages with mermaids in Papua New Guinea, 33 pages with the mysterious entities in Loch Ness and Lake Champlain, 16 pages with the putative sauropods (Mokele-Mbembe) of the Central African swamp-forest, and 15 pages with general definition and defense of cryptozoology as a legitimate subject for research. Clearly, if you want to be an interesting cryptobeast, you are well

advised to be a large vertebrate, and preferably human-like or dinosaur-like.

More than half the pages devoted to human-like creatures are reviews of five books. These books appear to deal mainly with second- and third-hand anecdotes; it seems generally agreed that the hard evidence — photographs, tapes, casts of footprints — is fraudulent. An article by Guoxing on 'The status of wildman research in China' gives an earnest account of available reports, and speculates on whether the Chinese wildmen are surviving descendants of *Ramapithecus*, *Australopithecus* or possibly *Gigantopithecus*. With commendable honesty, the article concludes "it should be emphasized that many of the participants in Wildman research in China are professional scientific workers. At the same time, however, it has to be admitted that most of them are not well trained in faunal ecology, primatology, vertebrate paleontology, paleoanthropology, physical anthropology and other disciplines concerned with this topic". Wagner recounts tales told him by New Guinea natives about the mermaid-like *ri*, and comes to three conclusions: "they are certainly not dugongs" (it is sometimes suggested that manatees or dugongs gave rise to sailors' stories about mermaids); the reports are more substantial than the 'inventories of rather fanciful 'bush' creatures' familiar to anthropologists working in New Guinea; and the reports "strongly suggest that some such creature exists, and that it remains unknown to science".

The papers on dinosaur-like creatures are, on the whole, less silly. A research report by LeBlond employs a photograph of the 'Lake Champlain monster' taken by Mansi in 1977, and uses standard results relating the appearance of the sea surface to wind speed, and thence to the length of wind waves, to estimate that the length of the 'monster' visible in the photograph is in the range 5–17 m. The evidence reviewed in other articles in *Cryptozoology* and in the pages of *Nature* over the past few years sug-

gests that large objects of some kind (logs look like the best guess to me) are present in deep lakes such as Loch Ness or Lake Champlain, and LeBlond's elegant estimate of the physical dimension of one such object is useful.

Mackal, Greenwell and Wilkinson recount persistent reports of Mokele-Mbembe, a large creature with a long flexible neck and a tail like an alligator's, which inhabits the 60,000 square mile Likouala swamps in the remoter parts of the People's Republic of the Congo. These investigators recorded stories from the native inhabitants, including one of a hunter who had tracked a creature that left a path of grass flattened as if by a great tail, and positive reactions to photographs of sauropods. The reports also suggested that the apple-size fruit of the molombo (a tropical vine found in Africa) were the main diet of the Mokele-Mbembe. A separate paper by Weber, Berry and Greenwell therefore analyses the nutrient content of these fruit, and compares it with the nutrient requirements of typical large mammals, to conclude that molombo fruit alone could not sustain the Mokele-Mbembe. Mackal and his collaborators sensibly conclude that "our expedition found no compelling evidence that Mokele-Mbembe exists", although they append the usual appeal for further investigation.

Many people feel that the correct response from a scientist to the kind of enterprise this journal represents should be one of friendly, even encouraging, tolerance. At very least, I am persuaded such may be an expedient image to project. We would be ill-served if everyone studied safe subjects. But, in truth, my reaction to *Cryptozoology* is regret for the money libraries will waste on acquiring the journal (if only as a curiosity) and regret for the dissipated efforts that could be directed more productively to studying some of the species of tropical plants, insects and other organisms that may be going extinct at a faster rate than they are being classified. Even on its own Alice-Through-The-Looking-Glass terms, the journal *Cryptozoology* is marked by a rather arbitrary focus on only two groups — human-like and dinosaur-like — from the full cryptozoological spectrum of animals that may or may not exist; this spectrum is catalogued by Cohen in *The Encyclopedia of Monsters* (Dodd, New York, 1983). Some of the omissions are understandable: for instance, the lack of work on the unicorn is undoubtedly because these beasts are fairly generally agreed to have been hunted to extinction, a process made easier by virtue of their maladaptive attraction to virgins. Less understandable — especially to an antipodean — is the absence of any work on the bunyip, a particularly engaging but elusive member of the Australian cryptofauna. □

Robert M. May is Class of 1877 Professor of Zoology at Princeton University, Princeton, New Jersey 08544.

Carbon cycle

New data upset ice age theories

from Philip Campbell

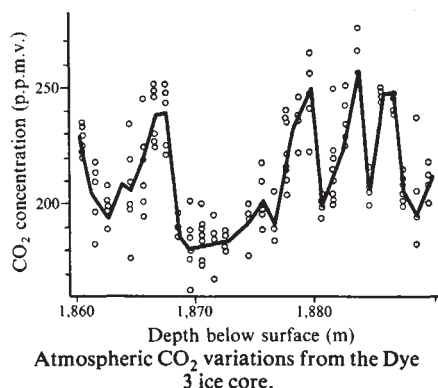
"It was the best of times, it was the worst of times... it was the epoch of belief, it was the epoch of incredulity..."

At the risk of over-dramatization, the opening lines of Dickens' *A Tale of Two Cities* reflect something of a recent meeting* devoted to the carbon cycle. Little of the meeting dealt with the effects of fossil fuels, the participants encountering enough problems and surprises from the natural cycle before it was perturbed by man. In particular, they were confronted with natural rates of change in atmospheric CO₂ concentrations much faster than previously measured, new data from deep-sea cores indicating that CO₂ variations precede changes in the extent of ice sheets (contrary to prevailing ideas), and theoretical results suggesting that atmospheric CO₂ was to a significant extent responsible for the warmth of Cretaceous climate.

A year or two ago, ice cores yielded the surprising result that at the end of the ice age, between 15,000 and 10,000 yr ago, the atmospheric CO₂ abundance climbed from about 210 to about 280 parts per million by volume (p.p.m.v.) in only a few thousand years^{1,2}. W.S. Broecker³ pointed out that over time scales shorter than the 180,000-yr residence time of CO₂ in the atmosphere and oceans, atmospheric CO₂ is controlled by the physical, biological and chemical state of the oceans. Thus such a 'rapid' increase of 70 p.p.m.v. would seem to have required an unusual perturbation to this system. Broecker suggested that detritus from surface organisms, containing organic carbon and phosphate, was deposited on the continental shelves as they were submerged following the melting of ice sheets. This reduced the phosphate/carbon ratio in the oceans and, phosphate being a limiting nutrient, the fixation rate of carbon by marine photosynthesis would have decreased. In this way the CO₂ concentration in ocean surface water, and hence the atmospheric CO₂ content, would have increased by the amount and in the time scale revealed by the ice cores.

A number of results presented at the conference were consistent with this model. For instance, one of its predictions is that, as a result of the removal of organic carbon from the ocean into shelf sediments, the deeper ocean would have become less prone to dissolve carbonate sediments, leading to enhanced preservation of CaCO₃. L.C. Peterson and W.L. Prell (Brown University, Rhode Island) sampled a series of deep-sea piston cores raised from the eastern flank of the Ninety-East Ridge in the east

Indian Ocean. The depth range over the flank from 2.9 to 4.4 km encompasses today's lysocline, the oceanic boundary below which foraminiferal tests, or shells, will dissolve. Over the 700,000 yr record, both ¹⁸O/¹⁶O isotope ratios — interpreted as a record of global ice volume — and the proportion of carbonate revealed clear periodicities of 100,000, 40,000 and 19–23,000 yr. According to the now-respected Milankovich theory, ice ages are driven at these periodicities by changes, respectively, in the eccentricity of the Earth's orbit, the tilt and the precession of the Earth's rotation axis. More to the



point, the amount of CaCO₃ present in the cores was a maximum when ice volume was decreasing most rapidly and was a minimum when the ice sheets were expanding at their quickest. This specific relationship concurs with a recent numerical model⁴ based on the phosphate-extraction hypothesis. A correlation of sea level falls and increased carbonate dissolution in the Canary Basin, North Atlantic, was also reported by J.T. Crowley (NSF Climate Program, Washington) using a similar experimental approach.

The theoretical impetus provided by Broecker's model was illustrated by several computer models of the ocean-atmosphere carbon cycle. F. Knox and M. McElroy (Harvard University) and J.R. Toggweiler and J.L. Sarmiento (Princeton University) described similar models in which the atmosphere, the intermediate/deep ocean, low-latitude and high-latitude shallow ocean reservoirs are represented by distinct boxes, linked by appropriate carbon fluxes. The purposes of these models is to investigate the roles of high-latitude nutrients and of ocean circulation rates in controlling atmospheric CO₂ levels, bearing in mind that restricted areas of the ocean surface, such as the Antarctic upwelling regions where productivity in surface waters is high, can have a considerable effect on the carbon chemistry of the entire deep oceans and the atmosphere. Both models confirm that an increase in

high-latitude surface nutrients is associated with enhanced atmospheric CO₂ levels, demonstrating that changes in large-scale (thermohaline) circulation and in high-latitude surface water productivity (caused, for instance, by changes in light levels) could have a crucial role. A 1,000 yr response-time of the predicted changes is imposed by the circulation time of the deep ocean.

All the results described so far have been consistent with and/or stimulated by the phosphate-extraction hypothesis. It is intriguing, to say the least, that two papers were presented that between them — as Broecker (Lamont-Doherty Geological Observatory, New York) himself pointed out — seem to demolish shelf deposition models.

The first challenge has come from ice cores. B. Stauffer, U. Siegenthaler and H. Oeschger (University of Bern) reported measurements of past CO₂ concentrations from the Dye 3 site in Greenland — a record spanning the period 25,000–40,000 yr ago. Four sharp peaks in CO₂, reaching levels of 250 p.p.m.v. from a 'background' of 200 p.p.m.v., occurred during the last ice age (see the figure). The magnitude of the changes are of the order which the phosphate-extraction model seeks to explain, but the excursions appear to have occurred within a hundred years or so — ten times faster than can possibly be explained by shelf deposition. The excursions are well correlated with oxygen isotope changes, as are accompanying variations in trace chemicals Cl⁻, NO₃⁻ and SO₄²⁻ (R.C. Finkel and C.C. Langway, SUNY, Buffalo). Stauffer *et al.* proposed that climate behaved as a bistable oscillator during the last glaciation. If the rapid CO₂ variations are also found in Antarctic ice cores, then the idea that CO₂ acts as an interhemispheric climatic amplifier will be greatly strengthened.

T. Wenk, Siegenthaler and Oeschger (University of Bern) described a box model constructed on identical principles to the two already discussed to explain these rapid variations. They reported that a 50% reduction from present-day conditions in the exchange flux between high-latitude (Antarctic) surface and deep waters results in a decrease in atmospheric CO₂ levels of 50 p.p.m.v., an effect which is enhanced if the large-scale circulation is increased. These authors maintain that changes in surface circulation could account for rapid CO₂ variation, although this view was challenged by other box-modellers. The role of deep-ocean circulation as an inhibitor of such rapid variations was left unresolved; all three box models were steady-state calculations, and models of the transient responses are now required.

A second nail for the coffin of shelf-deposition models was provided by N. Shackleton (University of Cambridge) and N.G. Pisias (Oregon State University), who presented high-resolution oxygen and carbon isotope records from an equatorial Pacific deep-sea core. Because marine

*The Chapman Conference on 'Natural variations in carbon dioxide and the carbon cycle' was held in Tarpon Springs, Florida on 9–13 January.

photosynthesis preferentially extracts ^{12}C from surface waters, the $^{13}\text{C}/^{12}\text{C}$ ratio in foraminifera can indicate changes in the oceanic carbon reservoirs. (It is essential to distinguish between this effect and that of deep-ocean circulation, which causes a progressive isotopic lightening of dissolved CO_2 in deep waters as they circulate through the rain of organic detritus from surface regions. Several talks at the meeting highlighted different ways of tackling this problem.)

Broecker had already showed³ that the difference in carbon isotope ratios in surface and deep waters varies with atmospheric CO_2 levels. Interpreting planktonic and benthic foraminiferal isotope ratios in this manner, Shackleton and Pisias provided a record of ice volume and atmospheric CO_2 over a 340,000 yr period. The Milankovich frequencies were clearly present with the CO_2 record having a stronger high-frequency content — consistent, perhaps, with the ice-core data. Both ice volume and CO_2 changes lag the orbital (Milankovich) changes but, most significantly, the changes in CO_2 lead those in ice-volume by, on average, 2.5×10^3 yr. This result is completely at odds with shelf deposition models (which predict that sea level changes lead CO_2 changes) and the authors concluded that variations in atmospheric CO_2 are part of the direct forcing of ice ages. They argued that changes in the ocean nutrient cycling and in ocean circulation must lie at the root of the CO_2 changes. However, the field is now open for more specific explanations.

This report has so far been restricted to variations in the carbon cycle over time scales of tens of thousands of years or less. But the Florida meeting covered the entire gamut of time scales up to 10^9 yr; those of 10^7 – 10^8 yr, associated with the Cretaceous and Cenozoic eras, were the subject of particularly lively discussion.

A provocative interpretation of South Atlantic deep-sea carbon isotope data from the last 75 Myr was presented by N. Shackleton. He used the ^{13}C record in bulk sediments to deduce changes in the partitioning between limestone and organic carbon and hence in the global organic carbon reservoir. According to his data, the amount of organic carbon stored in sediments 70 Myr ago exceeded that in today's oceans by about 10^{19} moles. Shackleton concluded that oxygen in the atmosphere must have been correspondingly enhanced over the present-day content by 20%; the dissolved oxygen concentration would have been similar to today's because of the reduced solubility in the warmer ocean waters at that time. H.D. Holland (Harvard University) and R.M. Garrels (University of South Florida) suggested, however, that the excess oxygen would have been accommodated by SO_4 in the oceans, and a debate ensued, hinging on the differences between the residence time of SO_4 in the ocean and the characteristic time scales

within Shackleton's data. J.R. Herring (US Geological Survey, Lakewood) maintained that the putative enhancement in oxygen is rebutted by the absence of a significant increase in charcoal fragments or other sedimentary signatures of natural plant burning.

Over the 100 Myr time scale, geological processes provide significant contributions to the carbon cycle. In particular, the balance between weathering, by which CO_2 is removed from the atmosphere and stored as carbonate, and metamorphism (in which the process is reversed) has a significant effect on atmospheric CO_2 . Recently a geochemical box model was published⁵ in which the oceans, the atmosphere, calcite, dolomite and Ca-Mg silicate reservoirs were linked by the chemical reactions of weathering and metamorphism. The novel aspect of the model lay in its treatment of the chemical rate constants. Those connected with weathering, for instance, were parameterized in terms of atmospheric CO_2 levels.

A number of problems and uncertainties associated with the published model were raised at the conference. However, these were trumped by its instigators, A. Lasaga, R. Berner and R. Garrels (Pennsylvania State, Yale and South Florida University respectively), who presented an extended model in which organic carbon, evaporates and pyrite reservoirs were incorporated. One of the model's central conclusions eventually remained unchallenged: that in

the Cretaceous, 100 Myr ago, the amount of CO_2 in the atmosphere was at least four, and maybe over ten times as great as today's, with average temperatures correspondingly elevated.

This conclusion was supported by S. Schneider, S. Thompson and E.J. Barron (National Center for Atmospheric Research). Using a three-dimensional atmospheric circulation model in which the continental geography resembles that of Cretaceous times, they found that no matter how oceanic circulation is varied, winter temperatures in high-latitude continental interiors remain near or below freezing, a situation belied by palaeobotanical evidence. They suggest that elevated CO_2 concentrations might have been the missing factor that produced the higher temperatures of that era.

These models and much of the rest of the work described in this report reflect one of the major themes to come out of the conference. As one of its organizers, E. Sundquist (US Geological Survey, Reston) emphasized, climate and the carbon cycle appear to have been more tightly linked than previously thought. □

Philip Campbell is Physical Sciences Editor of Nature.

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Evolutionary biology

When deviants are favoured: evolution of sex determination

from Paul H. Harvey and Linda Partridge

A NEW book from J.J. Bull¹ makes a fresh attempt at explaining the evolution of mechanisms that determine sex. What makes the problem a difficult one is the bewildering variety of sex-determining mechanisms found in the animal and plant kingdoms, and the seemingly chaotic way in which they are distributed among taxa. Even in species with separate sexes, male heterogamety (XY male, XX female) can be found in such diverse taxa as mammals, fruit flies and some vines, while female heterogamety (ZW female, ZZ male) occurs in birds, schistosomes and strawberries. Haplo-diploidy (males develop from unfertilized eggs and are therefore haploid, females from fertilized eggs and are diploid) is thought to have evolved at least eight² and probably more than twelve¹ times among insects, mites and rotifers. Sex may even be environmentally determined, as in many reptiles, a marine fish, an echiuroid worm, a crustacean parasite of fish gills, some nematodes and at least one species of amphipod.

Bull documents much of the diversity of

sex-determining mechanisms but his book differs from previous accounts because it specifically addresses the question of how one type of sex determination can evolve into another. His approach is to consider what combination of selective forces and historical constraints have resulted in the evolutionary changes observed, these being only a limited subset of the theoretically possible transitions. The application of this approach across a broad taxonomic range allows him to draw some very general conclusions about the parts played by selection and by evolutionary constraints.

Haplo-diploidy

Haplo-diploidy appears from its taxonomic distribution to have often evolved from an ancestral diploid condition, as does a functionally equivalent system — paternal genome loss — where males are produced from fertilized eggs which transmit only the maternally derived part of their genome to their offspring. What selective forces are responsible for the evolution of these systems? Hartl and Brown³ and Bull^{1,4} suggest that an allele

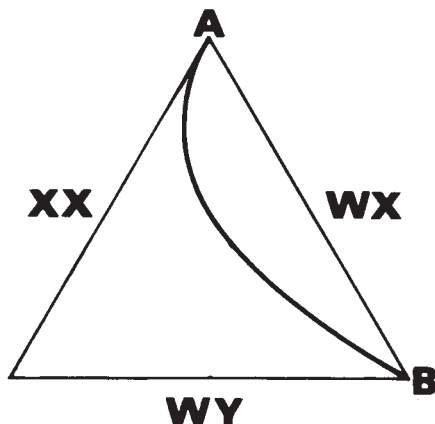
causing a female to produce haploid eggs that will develop into sons will increase in frequency in her grandchildren because haploid sons transmit maternal alleles to all their offspring whereas diploid sons transmit maternal alleles to only half their offspring. As long as the haploid sons leave more than one-half as many offspring as the diploid sons, haplo-diploidy will spread.

But this argument applies equally well to male and female parents — any genetic device in a parent that causes offspring to transmit only alleles from that parent should be favoured. Why, then, is it always the maternal genome that is transmitted? And why is the maternal genome always transmitted by sons rather than daughters? The first question is difficult to answer: selective transmission of the paternal genome could, in theory, be effected via haploid embryos that develop from sperm (which is improbable) or from a fertilized egg that lost its maternal genome. It is not yet clear why the latter mechanism has never evolved (as far as we know), partly because the mechanism of paternal genome loss is not well understood. Perhaps females are in a position to resist male-derived mutants that cause maternal genome loss. The reason that only sons transmit the maternal genome is probably related to the disadvantages of asexual inheritance⁵⁻⁷. Transmission by daughters would mean that male-derived alleles would never be transmitted to females and would cause asexual inheritance in the female line. Finally, haplo-diploidy could only arise from a diploid species with a sex-determining mechanism such that aberrant haploid individuals develop into males¹.

Genetic and environmentally determined sex

There have been several evolutionary transitions between genetic and environmentally determined sex. Environmental sex determination is likely to be favoured in a patchy environment where different patches affect the fitnesses of each sex differently, but where parent and offspring have little or no control over the type of patch in which the offspring develop⁸.

Take the case of mermithid nematode worms, for example. Free-living adult worms lay eggs in larval insect hosts: the worms hatch and grow to a size determined by the richness of the food source, which is itself determined by the density of the worms and the nutritional status of the host. But female fitness increases with size more than does male fitness and, as a result, selection has operated so that worms developing at high density or in a poorly fed host tend to become males, while those developing at low density or in a well fed host become females^{9,10}. In the case of these worms, offspring cannot choose the type of host in which they develop and mothers laying eggs may not be able to detect whether the potential host has already been parasitized by other mermithid nematodes. The two conditions



The curved line shows the equilibrium frequencies of the three different genotypes of female platyfish (see text). When the perpendicular distances from the three axes to any point on the line are scaled so that they sum to unity, their lengths give the frequencies of the three genotypes. The points labelled A and B represent extremes with female heterogamety (WY female, YY male) and male heterogamety (XY male, XX female). Populations at all other points on the line of equilibria contain three female genotypes (WY, XX, WX) and two male genotypes (YY, XY). (Refs 1, 17.)

of absence of patch choice and differential fitness of the two sexes thus apply. The model also seems to hold in other cases¹¹⁻¹³, and some preliminary data suggest it may well be relevant to environmental sex determination in reptiles^{14,15}.

Under what conditions can genes for sex determination invade species with environmentally determined sex? The theory given above suggests that changes in relative fitness of the two sexes must be fairly consistently associated with particular patch types for environmentally determined sex to be favoured. In the wild, this condition will not always hold. If mermithid nematode population density were to vary between seasons or if host nutritional condition also changed dramatically between seasons, then, under pure environmental sex determination, some seasons might produce very high proportions of males and others would produce excess females. This would result in fluctuating fitnesses for individuals of each sex

and, in such conditions, genotypic sex determination can invade¹. (Could some dinosaurs have gone extinct because their sexes were environmentally determined? A sudden change in climate without an appropriate genetic response could provide the conditions for producing populations consisting totally of frustrated males, or females if you will.)

Male and female heterogamety

Although there are conditions in which one sex-determining mechanism can invade a population with an alternative method of sex determination, this does not necessarily mean that the new mechanism will completely replace the old. Indeed, it is possible for two or more sex-determining mechanisms (or sex factors) to be maintained at equilibrium in the same population. Sometimes, instead of a single equilibrium, there is a continuous set of stable equilibria which can be described graphically by lines of 'neutral equilibria' along which any point describes a combination of equilibrium sex factor frequencies. When populations lie on such a line, there is a prospect for indeterminate evolution — genetic drift can carry small populations along the line of neutral equilibria. The commonest mechanisms of sex determination in animals — male and female heterogamety — could evolve from one another in this way.

An example is provided by a tropical poeciliid fish, the platyfish (*Xiphophorus maculatus*), a favourite among aquarium fanciers, of which the earliest captive individuals proved to have female heterogamety while later collections had male heterogamety. It turns out that natural populations are polymorphic for three sex factors (W, X and Y) which could occur in six different combinations: XY, YY, XX, WX, WY and WW. The XY and YY genotypes usually develop as males and XX, WX and WY become females¹⁶; WW genotypes do not in fact occur because male gametes can never bear W factors. Assuming that fitnesses of the different genotypes are the same within each sex, their equilibrium frequencies can be calculated¹⁷ (see the figure). At one



100 years ago

PROF. HULL, who has returned with his party, brings with him, it is stated, materials for the construction of a geological map of the Holy Land very much in advance of anything which could hitherto be attempted. He is reported to have traced the ancient margin of the Gulfs of Suez and Akaba to a height of 200 feet above their present levels, so that, according to Prof. Hull, the whole country has been submerged to that extent, and has been gradually rising. As one result of this rise, the Professor is of opinion

that at the time of the Exodus there was a continuous connection of the Mediterranean and the Red Sea. As regards the Dead Sea, Prof. Hull believes he has discovered that it formerly stood at an elevation of 1400 feet above its present level — that is to say, 150 feet above the level of the Mediterranean. He believes he has also found evidences of a chain of ancient lakes in the Sinaitic district, and of another chain in the centre of the Wady Arabah, not far from the watershed. The great line of the depression of the Wady Arabah and the Jordan Valley has been traced to a distance of more than a hundred miles. The materials for working out a complete theory of the origin of this remarkable depression are stated to be now available. Prof. Hull has in hand, besides his scientific report, a popular account of his journey, which will first appear in the *Transactions* of the society. From *Nature* 29, 389, 21 February 1884.

extreme, X factors disappear from the population and female heterogamety (WY females, YY males) describes the system (point A). At the other extreme the W factor disappears, leaving a system of male heterogamety (XX females, XY males; point B). Platyfish populations can thus be viewed as in transitional states between systems of either male or female heterogamety. Environmental change that differently affects the fitnesses of the various genotypes within each sex could trigger transitions between the two modes of sex determination.

Hermaphroditism to heterogamety

Just why it is that male heterogamety is common and female heterogamety rare¹⁸⁻²⁰ among dioecious plants (those having separate sexes) has been explained elegantly by Charlesworth and Charlesworth²¹. Their argument necessarily supposes that dioecious plants evolved from hermaphrodite ancestors through an intermediate stage, of either gynodioecy (females and hermaphrodites) or androdioecy (males and hermaphrodites). A transition from hermaphroditism to either gynodioecy or androdioecy may be favoured if loss of one sexual function can be balanced by increased resource allocation to the other, and if the single-sex individuals avoid self-pollination with its attendant inbreeding depression. But pollen from the male plant of an androdioecious species may be at a disadvantage in gaining access to the ovules of the hermaphrodites in competition with the hermaphrodites' own pollen. By contrast, females in a gynodioecious species do not have this problem because hermaphrodites produce more than enough pollen to fertilize all available ovules. Gynodioecy may therefore evolve more readily, and this may be one reason why androdioecy is much rarer in nature than gynodioecy²²⁻²⁴.

From gynodioecy one further evolutionary

step can be taken to male heterogamety. It is assumed that females of gynodioecious species are homozygous recessives — for they will have lost male function through mutation, and most mutants are recessive. This makes it possible for full dioecy to evolve, though it can only be achieved by way of a dominant mutation that produces female-sterile (that is, male) plants, replacing the hermaphrodites²¹. Mutations at loci that are genetically closely linked to the male-sterility locus will be favoured over those that are not because they will be less likely to allow male-sterile and female-sterile plants to segregate in the population. As a dominant mutation is required to produce males, it is male heterogamety that will evolve from gynodioecy; and as gynodioecy is more common than androdioecy, the higher frequency of male heterogamety

is thus explained.

The processes that lead to transitions between different systems of sex determination have received erratic attention from evolutionary biologists in general and population geneticists in particular. Bull's book¹ provides a general analytical framework for studying these processes, a comprehensive review of a relevant but scattered literature and a focus on unanswered questions. It also makes an excellent complement to Charnov's recent book on sex allocation theory²⁵, which considers how individuals should partition resources into male and female function²⁶. □

Paul H. Harvey is in the School of Biological Sciences at the University of Sussex, Brighton BN1 9QG, and Linda Partridge is in the Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT.

Ions caught in their tracks

THE cloud chamber, once described by Lord Rutherford as "the most original and wonderful instrument in scientific history", dominated nuclear physics for half a century. It was invented almost inadvertently by C.T.R. Wilson when he sought to duplicate in the laboratory the optical effects produced by the interplay of clouds and sunlight encountered on a climbing holiday in Scotland in 1894. To generate artificial mist, Wilson designed a chamber in which to expand moist air and found that mist formed above a critical value of the expansion. Experimenting with the X rays discovered by W.C. Röntgen in 1895, he discovered that below that value the passage of the rays was rendered visible by a condensation track formed from the supersaturated air. He had in effect constructed a particle detector.

The design was progressively modified and improved, and in 1911 Wilson was ready to record the transitory tracks photographically. With a small cylindrical chamber activated by a piston from below, it was possible to record clearly the droplets condensing along the tracks not only of X rays but also of α , β and γ rays. Wilson concluded that the tracks "of ionising rays

of any kind through a moist gas may be made visible by condensing water upon the ions set free".

The accompanying photograph, taken at the Cavendish Laboratory in 1911 by the light of a mercury spark, was the first published in Wilson's historic paper 'On a method of making visible the paths of ionising particles through a gas' (*Proceedings of the Royal Society A* **85**, 285; 1911). A potential difference of 8 volts was applied between roof and floor of the chamber. The camera was fitted with a Beck 'isostigmat' lens at $f/5.8$ and loaded with an Ilford Monarch plate. The shower of tracks traces the passage of α particles — helium nuclei — from a dot of radium on the tongue of a spintharoscope (left). "The very beautiful sight of the clouds condensed along the tracks of the α particles was seen for the first time", recalled Wilson in his Nobel lecture of 1927. The photograph is reproduced by courtesy of the Science Museum, where the 1912 version of the Wilson cloud chamber is preserved.

Jon Darius

Selected from *Beyond Vision* by Jon Darius of the Science Museum (to be published in April by Oxford University Press).



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Chemistry

Chemically generated spatial structures

from Irving R. Epstein

It might seem self-evident that a pattern formed by inhomogeneities in a liquid will eventually blur and disappear through the action of diffusion and that a liquid that is homogeneous will remain so. But recent work in nonlinear chemical dynamics is providing a set of extraordinary examples that do not conform to the intuitively attractive notion that spatial structures tend to decay rather than grow. These phenomena and the models proposed to describe them are beginning to have an impact not only in chemistry but in biology and geology as well, though some of the models are still highly controversial.

Probably the most venerable spatial structures seen in solutions are the Liesegang rings, known since the turn of the century¹. Two solutions containing a pair of ions, such as silver and dichromate, which form a sparingly soluble compound, are placed at opposite ends of a tube containing a gel. As the ions diffuse towards one another, precipitate appears not, as one might expect, uniformly, but in a series of sharp nearly periodically spaced rings. Theories recently developed by Ross², Ortoleva³ and co-workers challenge a 60-year old view⁴ that the phenomenon results from the build-up and release of supersaturation. These new theories successfully explain Liesegang's as well as newer and more puzzling experiments by invoking the competitive autocatalytic growth of different sizes of colloid particles coupled to diffusion of the reactants. Ortoleva⁵, by applying similar ideas, but on much larger scales of time and length, is able to explain the development of textural patterns in initially unbanded metamorphic rock — although the approach has met with some resistance from geologists.

Particularly exciting visually is the spontaneous development of trigger wave or bull's eye patterns (see the figure) in an initially homogeneous disc of reactant liquid. Winfree⁶ has developed recipes and techniques for producing spirals and scroll waves as well as the simpler patterns, and Krinsky⁷ has been able to generate multi-armed vortices (see the figure). A recent paper in *Nature* describes how to add a third dimension to these structures⁸, while in this issue (p.717), Avnir and Kagan describe structures generated at membrane surfaces. Early studies of these phenomena were limited to a single chemical system, the Belousov-Zhabotinsky (BZ) oscillator, but De Kepper *et al.*⁹ have shown that the same patterns may be observed in systems with quite different chemistry. The resemblance between these chemical

structures and those seen in the aggregating slime mold *Dictyostelium discoideum*¹⁰ are striking.

These patterns may be understood in a general sense as the result of a dynamic instability arising from the coupling of a chemically excitable system with diffusive transport and a recent computer simulation based on a two-parameter model¹¹ yields patterns remarkably like those found in the BZ reaction. However, a convincing calculation that predicts the wave pattern from a plausible chemical mechanism for a system as complicated as the BZ reaction has yet to appear. A first step in that direction has been taken by Showalter and colleagues¹², who treat a simpler phenomenon, the propagation of a single concentration wave in the arsenite-iodate reaction, by adding one-dimensional diffusion terms to a plausible model for the chemistry. Both the shape and the velocity of the wave are accurately predicted.

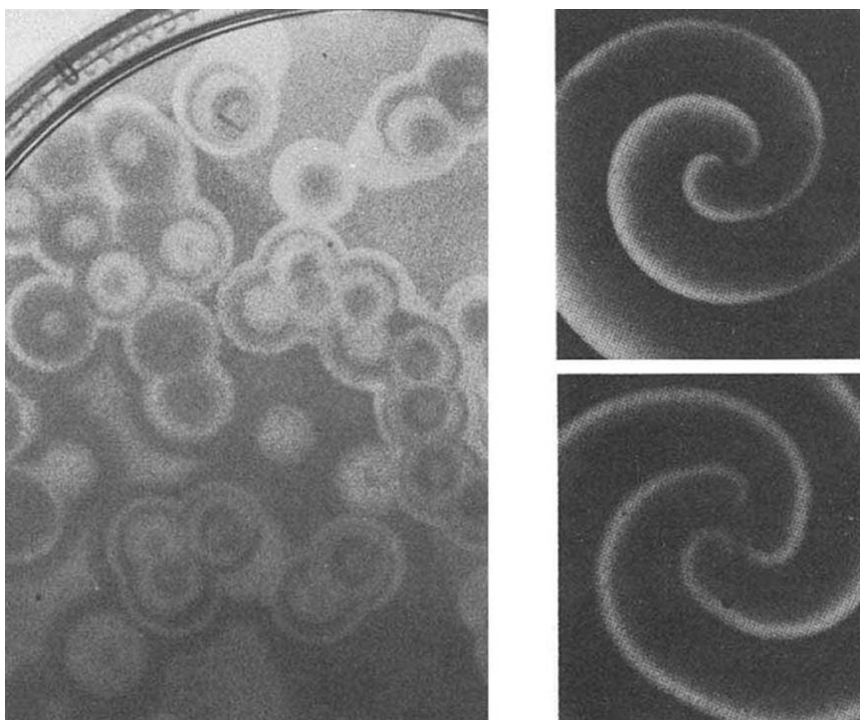
One rather controversial class of spatial phenomena is generated photochemically when initially homogeneous solutions are illuminated. Two recent papers^{13,14} have refuted earlier claims of periodic temporal oscillation in the light emitted by certain photochemical systems subject to constant illumination. Instead, the observed

behaviour is shown to result from spatial oscillations generated by convective motion of the fluid in response to temperature gradients set up by the illumination. (A related controversy involves the report published in *Nature* a few months ago¹⁵ that previously published photochemically generated spatial structures^{13,16}, too, result from convection rather than photochemistry.)

Although the study of spatial pattern formation in chemical systems is still in its infancy, it is progressing rapidly. Answers to the questions raised by some of these beautiful and often counterintuitive phenomena promise important insights not only into chemical dynamics but into such biologically significant problems as morphogenesis as well. □

Irving R. Epstein is Professor and Chairman of Chemistry at Brandeis University, Waltham, Massachusetts 02254.

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Left, spatial wave pattern arising spontaneously in an initially homogeneous mixture of sodium iodide, sodium chlorite, malonic acid, sulphuric acid and starch indicator. Right, wave movement in two- and three-armed vortices (from ref. 7).

Calcium-activated potassium channels and their role in secretion

Ole H. Petersen & Yoshio Maruyama

MRC Secretary Control Research Group, The Physiological Laboratory, University of Liverpool, PO Box 147, Brownlow Hill, Liverpool L69 3BX, UK

Single-channel recording by the patch-clamp technique has now characterized three kinds of membrane potassium channels activated by intracellular calcium ions in animal cells. These play a crucial part in the regulation of membrane potential and of secretion.

THE concentration of potassium ions (K^+) in cells relative to extracellular space is normally maintained either directly by the ATP-dependent sodium-potassium (Na^+-K^+) pump in the plasma membrane or, indirectly, by co-transport processes ultimately driven by the same mechanism¹⁻³. The exit of K^+ from cells, on the other hand, is mediated by trans-membrane K^+ channels of which there are several kinds⁴. The K^+ channels whose activity is regulated by intracellular calcium ion (Ca^{2+}) concentration⁵⁻⁷ are of particular interest because they suggest explanations of several well-known phenomena in which the export of K^+ from cells appears to be linked with changes of cell metabolism and membrane potential. These include the adrenaline-induced increase in plasma K^+ concentration (due to K^+ exit from liver cells)^{8,9}, salivary gland K^+ transients (due to K^+ exit from acinar cells)^{10,11} and the 'Gardos effect' in erythrocytes (K^+ release from red cells)^{12,13}. In the nervous system these channels play a major role in regulating electrical activity¹⁴.

The advent of the patch-clamp technique for studying the behaviour of single trans-membrane channels¹⁵⁻¹⁸ has made possible the recognition of several categories of K^+ channels in the membranes of animal cells. In what follows, we shall be concerned only with the K^+ channels activated by Ca^{2+} , of which there appear to be three distinct types—two which are sensitive to membrane voltage but which differ in K^+ conductance, and which are known as the 'large' and 'small' voltage-activated channels, and a third which is insensitive to membrane voltage, unselective among cations but which nevertheless is activated by Ca^{2+} ions. The presence of K^+ channels activated by Ca^{2+} has already been demonstrated in a variety of tissues from several species by means of single-channel recording^{5-7,19-29}. Individual cells may contain more than one type of Ca^{2+} -activated K^+ channel^{19,20}.

The large K^+ channel

The existence of K^+ -channels which are sensitive to the internal free Ca^{2+} concentration ($[Ca^{2+}]_i$) within the cell has been demonstrated only in the past few years by means of the patch-clamp technique (Table 1). That some of these channels have very high conductance was first demonstrated by Marty⁶ in the membranes of cultured chromaffin cells. Conductances as great as 200 pS or more have been found in various cell membranes, and channels of this type are activated by membrane depolarization. Marty noted two effects of Ca^{2+} at the inner surface of the membrane—increasing probability that high-conductance channels will open on membrane depolarization and a modest decrease of conductance in open channels. While the first effect has been amply confirmed^{7,20-23}, the reported decrease of channel conductance with increasing $[Ca^{2+}]_i$ has not always been found^{20,21,23}.

The sensitivity of high-conductance K^+ channels to $[Ca^{2+}]_i$ seems to vary considerably from one preparation to another.

Marty's original observations with cultured chromaffin cell membranes suggest that depolarization provokes full channel opening at a Ca^{2+} concentration of 10^{-8} M. At the same time, however, depolarization of inside-out patches of membrane from mammalian salivary glands evokes marked activation of these channels when the inner surfaces are exposed to nominally Ca^{2+} -free solutions containing 1 mM EGTA (ref. 20). By contrast, in cultured rat muscle cells, depolarization from -50 to $+50$ mV has been shown not to evoke channel opening at 10^{-8} M Ca^{2+} , to have virtually no effect at 10^{-7} M but to require internal concentrations of 10^{-6} M for channel activation²¹. While it seems that the high-conductance channels of the rat muscle preparation are Ca^{2+} -dependent (see ref. 21), in general it may be more appropriate to use the term ' Ca^{2+} -activated', since depolarization can open the channels in the absence of Ca^{2+} in many preparations²⁰.

In secretory cells such as the chromaffin and the salivary and pancreatic acinar cells, it is clear that the K^+ channel is strictly under the dual control of membrane potential and $[Ca^{2+}]_i$ ^{6,20,23}. Under physiological conditions, an increase in $[Ca^{2+}]_i$ will increase the open state probability (switch on the channel), which will increase the membrane potential and so, in turn, reduce the probability of channel opening. Thus, K^+ exit is kept in good control. The Ca^{2+} sensitivity appears appropriate for physiological regulation since a change in $[Ca^{2+}]_i$ from 10^{-8} to 10^{-7} M causes a marked increase in open state probability and an increase to 10^{-6} M causes near maximal opening of the K^+ conductance pathway²³.

The conductance of the K^+ channel is very high. In symmetrical high- K^+ solutions, values of 180 to 250 pS have been obtained^{6,20,23} (Table 1). The variations of conductance between different tissues are, however, puzzling. In our laboratory we generally find that the K^+ channels in pig pancreatic acinar cells have a conductance of about 200 pS whereas in the salivary glands of mice and rats, the channels have a conductance of about 250 pS. The possibility of sub-states of the channel has been raised by observations of smaller channels of varying conductances when membrane patches are artificially exposed to symmetrical high K^+ solutions^{20,23}, but detailed investigations of these smaller current steps have not been undertaken.

In spite of the high unit conductance, the K^+ channel is very selective. The channel discriminates between the closely related alkali metal ions K^+ and Rb^+ . With extracellular Rb^+ and intracellular K^+ , K^+ current can easily be made to flow from the inside to the outside, but no inward Rb^+ current can be evoked. With the Rb^+/K^+ gradients reversed, inward K^+ current, but not outward Rb^+ current, can be observed (Y.M., D. V. Gallacher and O.H.P., unpublished data). It is important to be aware of this rather extreme selectivity as ^{86}Rb is frequently used as a tracer in flux experiments to assess K^+ permeability since it has a much longer half-life than ^{42}K (ref. 2).

The K^+ conductance of the plasma membrane depends not only on the single-channel conductance and the probability of

Table 1 Properties of Ca^{2+} -activated channels permeable to K^+ in various preparations as determined by patch-clamp method

Tissue	Maximal single-channel conductance (pS)	Ion selectivity	Voltage dependent	Channel density	Refs
Neurones:					
<i>Helix pomatia</i>	19	K^+ channel	Yes	?	5
Bullfrog sympathetic ganglia	100	K^+ channel	Yes	?	70
Mouse neuroblastoma	22	Non-selective monovalent cation channel	No	?	19
Muscle:					
Rat myotubes	187,220	K^+ channel	Yes	?	7, 21
Rabbit T-tubule	226	K^+ channel	Yes	?	71
Rat heart	35	Non-selective monovalent cation channel	No	?	27
Endocrine glands:					
Bovine chromaffin cells	180	K^+ channel	Yes	?	6
Rat anterior pituitary cells	208	K^+ channel	Yes	?	22
Exocrine glands:					
Mouse parotid acini	250	K^+ channel	Yes	?	20
Mouse submandibular acini	250	K^+ channel	Yes	?	20
Rat parotid acini	250	K^+ channel	Yes	?	20
Pig pancreatic acini	200	K^+ channel	Yes	50 per cell	23
Mouse pancreatic acini	30–35	Non-selective monovalent cation channel	No	?	28, 29
Rat pancreatic acini	30–35	Non-selective monovalent cation channel	No	?	28, 29
Erythrocytes:					
Human red cells	18, 17	K^+ channel	?	?	24, 25
Frog red cells	18, 50	K^+ channel	No	?	26

channels being open at any time, but also on the channel density. The number of channels in a cell can be estimated by a combination of patch-clamp single-channel and whole-cell current recording if the K^+ channel is the only channel of quantitative importance for the conductance of the plasma membrane in the whole cell. This condition seems fulfilled in the pig pancreatic acinar cells. In the resting condition, where $[\text{Ca}^{2+}]_i$ lies between 10^{-8} and 10^{-7} M there are about 50 large K^+ channels per acinar cell²³.

The small K^+ channel

Very few patch-clamp investigations of this channel, which may or may not be related to the possible substates of the large K^+ channel mentioned above, have been carried out and relatively few details are therefore known. Lux *et al.*⁵ described a 19 pS Ca^{2+} -activated K^+ channel in *Helix pomatia* neurone which is switched on much more slowly than the large 200 pS channel, described above, following depolarization. A K^+ channel with a similar conductance (18 pS) is found in human red cells^{24,25} but only after their treatment with iodoacetic acid and adenosine (Gardos treatment¹²). It has recently been shown that the small K^+ channel as well as a somewhat larger K^+ channel (50 pS) in frog red cells can be activated by internal Ca^{2+} (refs 25, 26).

Non-selective cation channels

Not all Ca^{2+} -activated channels are selective. A Ca^{2+} -dependent non-discriminatory cation channel with a conductance of about 30 pS at room temperature (the conductance of this channel is very temperature sensitive) was first described by Colquhoun *et al.*²⁷. In marked contrast with the large K^+ channel, the non-discriminatory channel is virtually voltage-insensitive. It is a truly Ca^{2+} -dependent channel in the sense that opening and closure of the channel cannot be observed in the absence of Ca^{2+} on the inside of the membrane. Such channels have subsequently been found in mouse neuroblastoma cells¹⁹, mouse and rat pancreatic acinar cells^{28,29} and rat thyroid follicular cells (D. Moor and Y.M., unpublished observation). The channel is virtually impermeable to Cl^- (refs 19, 27, 28) and is almost

equally permeable to all the alkali metal ions¹⁹. Permeability for Ca^{2+} has not been directly demonstrated^{19,30}, is certainly low, but may not be entirely insignificant³¹. The precise relationship between the open state probability of the channels and $[\text{Ca}^{2+}]_i$ is not yet known. We have found that channel opening can be observed at $[\text{Ca}^{2+}]_i = 10^{-7}$ M immediately after excision of a pancreatic acinar membrane patch into the inside-out conformation but that after 10–20 s, higher (eventually micromolar) Ca^{2+} concentrations are required to evoke channel opening (Y.M. and O.H.P., unpublished data). The fact that the responsiveness of channels to $[\text{Ca}^{2+}]_i$ decreases with the passage of time suggests that the commonly reported observations^{19,27–29} that the channels are insensitive to Ca^{2+} may not describe the behaviour of these unselective channels in intact cells. It is relevant that the pancreatic secretagogue cholecystokinin (CCK) has been shown indirectly to activate the non-discriminatory channel in intact pancreatic acinar cells via internal Ca^{2+} (ref. 29).

Role in secretion

From a biophysical point of view, two types of secretory cells can be distinguished: those with and those without the ability to conduct electrical action potentials. In general, epithelial cells seem not to fire action potentials whereas most endocrine cells do. (There are, however, exceptions to this rule³².)

Endocrine cells: In the endocrine cells the K^+ channel acts as a link between $[\text{Ca}^{2+}]_i$ and the membrane potential. In typical endocrine cells, such as the chromaffin and the pancreatic β -cells as well as in nerve endings, Ca^{2+} needed in excitation-secretion coupling enters the cells through specific voltage-gated Ca^{2+} channels^{33–37} which are opened by membrane depolarization and closed by hyperpolarization. Therefore, as the entry of Ca^{2+} produces an increase in $[\text{Ca}^{2+}]_i$, so the K^+ selective channels will be activated leading to membrane hyperpolarization and impedance of further Ca^{2+} influx. A particularly beautiful example of the interplay between Ca^{2+} influx, $[\text{Ca}^{2+}]_i$ and K^+ conductance resulting in an oscillating membrane potential pattern (depolarization—plateau with Ca action potentials—hyper-

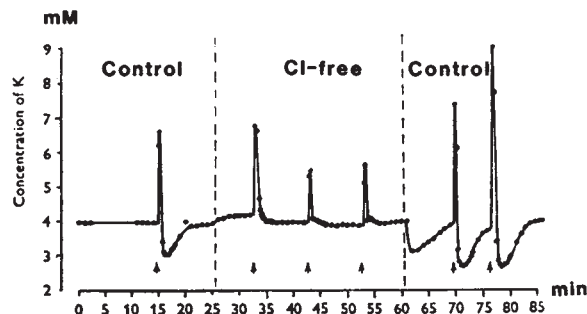


Fig. 1 Acetylcholine-evoked K^+ release and reuptake in perfused cat submandibular gland. The figure shows the concentrations of K^+ in the venous outflow from the gland in one typical experiment. The perfusion fluid flowing into the gland was a physiological saline solution containing 4 mM K^+ . Arrows denote injection of 10 μ g ACh into the arterial inflow to the gland. In the control periods the major salt component of the oxygenated perfusion solution was 140 mM NaCl whereas during the period labelled Cl-free it was 140 mM $NaNO_3$. Osmolarity, pH, K^+ and Ca^{2+} concentrations were kept constant throughout. Note that although the first ACh stimulation in the Cl-free period evokes a normal K^+ release the K^+ reuptake is virtually abolished. Cl- readmission evokes immediate K^+ reuptake. The fluid secretion responses to ACh during the Cl-free perfusion were about 30% of the values obtained in the second control period (from ref. 47).

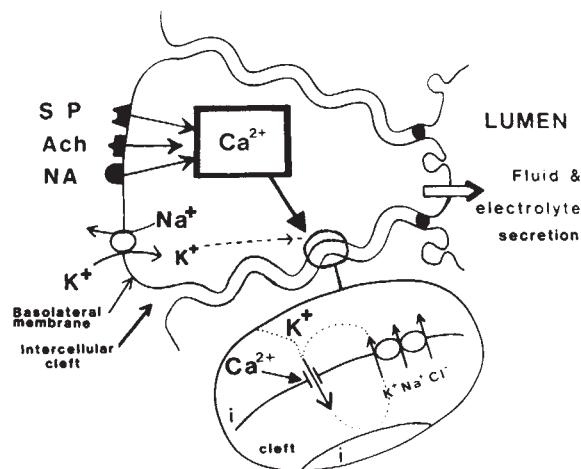


Fig. 2 Simplified scheme to account for control of electrolyte transport in baso-lateral plasma membrane of fluid secreting epithelia. The figure specifically represents part of a mammalian salivary gland acinus and its major secretagogues, substance P, acetylcholine and noradrenaline but with other secretagogues and/or various additions, the model could represent a wide range of secretory epithelia. In the proposed scheme, secretagogues first increase $[Ca^{2+}]_i$ (as suggested by a variety of evidence^{32,34,35,72-74} including a recent direct demonstration in mouse acinar cells⁷⁵). The increased $[Ca^{2+}]_i$ would open Ca^{2+} -activated K^+ channels in the baso-lateral membranes of the cell (such channels are well known^{20,23}, there is some evidence of their localization in the lateral plasma membranes⁶⁵ and a decrease in intracellular and increase in extracellular K^+ activity following stimulation have been demonstrated^{45,46}). K^+ released through the channels into the narrow intercellular clefts would be taken up again, primarily through a K^+ - Na^+ - Cl^- co-transport system. The rate of transport in the cycle of K^+ release and reuptake would be regulated at the K^+ channel by internal Ca^{2+} and would be directly linked to the rate of NaCl uptake by the cotransport system. In the salivary glands it is likely that part of the K^+ release occurs via the Ca^{2+} -activated non-selective cation channel²⁰ explaining why the intracellular Na^+ concentration rises during stimulation^{47,48} and part of the K^+ uptake could occur via the Na^+ - K^+ pump (but intracellular Na^+ activity measurements have not been carried out). The operation of the Na^+ - K^+ - Cl^- co-transport system is of course dependent on the Na^+ gradient and therefore ultimately on the operation of the Na^+ - K^+ pump. (Note that there is no evidence in regard to specific localizations of Na^+ - K^+ pump and co-transport system to basal and lateral membranes, respectively and the two transport proteins probably co-exist both in the lateral and basal plasma membranes.) The Na^+ - K^+ - Cl^- co-transport is probably electroneutral as the most likely stoichiometry is 1 Na^+ , 1 K^+ and 2 Cl^- (ref. 58).

polarization—gradual slow depolarization—depolarization...) was demonstrated several years ago by Gorman and Thomas in *Aplysia* neurones³⁸. Interestingly, as previously pointed out³², this pattern resembles very much that seen in mammalian pancreatic β -cells³⁹. Ca^{2+} -activated K^+ channels⁴⁰ can indeed be regarded as the crucial regulatory link between $[Ca^{2+}]_i$ and therefore insulin secretion, and Ca^{2+} influx^{32,41,42}. The insulin secretagogue glucose, according to one hypothesis, acts by decreasing the Ca^{2+} sensitivity of the K^+ channels, prolonging the plateau phase with the Ca^{2+} action potentials and thereby enhancing $[Ca^{2+}]_i$ (refs 32, 41, 42).

Exocrine cells: The role of Ca^{2+} -activated K^+ channels in the epithelial cells from which action (spike) potentials are absent must clearly be a very different one. The fluid-secreting epithelia of several tissues (salivary glands, sweat glands, lacrimal glands, the exocrine pancreas, the gastric mucosa and the tracheal epithelium) are under strict nervous and/or hormonal control. In many of these tissues it has been clearly shown that nervous or hormonal activation of secretion is associated with release of cellular K^+ (refs 32, 43). The salivary glands, which have been investigated in considerable detail, will be treated here as a model for other fluid-secreting epithelia.

Salivary gland acini secrete an isotonic plasma-like fluid at a low rate (if at all) in the resting condition but at an extraordinarily high rate during nerve stimulation⁴³. Secretion is Ca^{2+} dependent⁴⁴. Nerve stimulation or direct stimulation with acetylcholine, noradrenaline or substance P evokes loss of cellular K^+ (refs 32, 43). Both a marked decrease in intracellular K^+ and a marked increase in extracellular K^+ have been measured^{45,46}. The stimulant-evoked K^+ loss, which is due to electrodiffusion^{47,48}, can be evoked in the absence of neurotransmitter stimulation by the Ca^{2+} ionophore A23187 (ref. 49). The direct demonstration of the large Ca^{2+} -activated K^+ channel in the baso-lateral plasma membranes of several mammalian salivary glands explains the stimulant-evoked K^+ loss and the Ca^{2+} -involvement²⁰. Following release of K^+ from stimulated salivary glands, there is reuptake of K^+ . This has generally been assumed to be due to operation of the ouabain-sensitive Na^+ - K^+ pump and certainly K^+ reuptake (but not stimulant-evoked K^+ release) can be blocked in the presence of ouabain⁴⁷. However, as seen in Fig. 1, K^+ reuptake is also acutely and reversibly abolished when the Cl^- component of the perfusion fluid is replaced by NO_3^- (ref. 47) (similar results were obtained using sulphate replacement⁵⁰). Stimulant-evoked

secretion is markedly reduced under these conditions as first shown by Lundberg⁵¹. The K^+ reuptake and secretion are also acutely sensitive to reductions in extracellular Na^+ concentration⁴⁷. These findings could be explained by postulating linked K^+ , Na^+ and Cl^- inward movement through a carrier system (Fig. 2) of the type now characterized in red cells⁵²⁻⁵⁶, kidney cells⁵⁷⁻⁵⁸ and the intestine⁵⁹, but not known to exist at the time of the original observations^{47,51}. The involvement of a K^+ - Na^+ - Cl^- co-transport system in secretion from the salivary gland is also supported by Lundberg's observation that replacement of Cl^- in the perfusion fluid by NO_3^- , I^- or SCN^- but not by Br^- , reduced the secretory responses by 85–90% (ref. 60). This resembles the situation in erythrocytes where only Br^- can replace Cl^- in the operation of the co-transport system although all the other anions can be equilibrated across the membrane by the anion (Cl^- - HCO_3^-) exchanger^{52,55}.

The novel feature of the model proposed in Fig. 2 is that cycling of K^+ through channel and co-transport system allows NaCl uptake through the basolateral membrane of the cell and that the rate of NaCl uptake is directly linked to the rate of transport in the cycle of K^+ release and uptake. The point of

regulation has been clearly identified at the molecular level as the Ca^{2+} -activated K^+ channel²⁰. The ability of ouabain slowly to block the K^+ reuptake⁴⁷ is of course entirely consistent with the hypothesis proposed, since uptake through the co-transport system will be dependent on the presence of the Na^+ gradient. The model shown in Fig. 2 does not deal with the processes in the luminal membrane, but our hypothesis is not incompatible with the experimental results obtained on *Calliphora* salivary glands and many other tissues suggesting the presence of a luminal Ca^{2+} -activated Cl^- conductance controlling salt exit from the epithelium⁶¹⁻⁶³. Indeed it would seem desirable, perhaps even necessary, as suggested by Burgen⁶⁴, to have control of transport at both baso-lateral and luminal plasma membranes.

It may be questioned whether K^+ release through Ca^{2+} -activated K^+ channels is large enough to cause a marked change in the transmembrane K^+ gradient but in several salivary glands the changes have been shown directly to be very substantial (Fig. 1) (see also refs 11, 45, 46). The measurements of extracellular K^+ activity in cat and dog submandibular glands show maximal increases after nerve stimulation of 15–18 mM (ref. 45). The changes in K^+ activity along the lateral plasma membrane (narrow extracellular clefts between the cells³²) are, however, likely to be even larger. A recent voltage clamp study on mouse pancreatic acini indicates that the ion channels controlled by secretagogues mainly reside in the lateral membranes bordering on the narrow extracellular spaces⁶⁵. If this is generally true then nearly all the K^+ will be released directly into the intercellular spaces and the K^+ activity obtained at this site will clearly be higher than in the interstitial fluid at large.

The initial stimulant-evoked change in the transmembrane K^+ gradient is likely to provide a major stimulus for salt uptake via the co-transport system (explaining the very high initial secretory rates⁴³), but there is evidence that it is only short-lived⁴⁵. However, even in the steady state situation of sustained secretion, it is essential to maintain the open state of the K^+

channels as continued K^+ exit must balance the K^+ uptake via the co-transport processes since the primary acinar secretion has a low (plasma-like) K^+ concentration⁴³.

Future perspectives

Although detailed investigations of the properties of the Ca^{2+} -activated K^+ channels have been carried out on several different cell types, little is yet known of the mechanisms involved in modulation of the channels by hormones and neurotransmitters. Recent evidence from our laboratory suggests that the pancreatic secretagogue CCK, acting via an increase in $[\text{Ca}^{2+}]_i$, not only increases the open state probability of the large K^+ channel in pig pancreatic acinar cells but also rapidly (and reversibly) increases the number of K^+ channels per cell, (2–3-fold increase with 2×10^{-10} M CCK). The mechanism is unknown but the observations are reminiscent of those in which ADH and aldosterone have been shown rapidly to increase the density of amiloride-sensitive Na^+ channels in the toad urinary bladder^{66,67}. The role of protein phosphorylation in controlling K^+ conductance is also likely to demand further attention. Voltage-clamp studies of the Ca^{2+} -activated K^+ conductance in internally perfused snail neurones show an enhancement of the outward K^+ current after addition of the catalytic sub-unit of cyclic AMP-dependent protein kinase to the internal perfusing medium⁶⁸, but single-channel current studies are not yet available. The role of the Ca^{2+} -activated K^+ channels in secretion, in particular in epithelial cells, also needs to be further clarified. The availability of specific blockers for the different channels would be an enormous help, but satisfactory compounds have not been found. The possible physiological regulatory role of internal Na^+ blockade of the large Ca^{2+} -activated K^+ channel⁶⁹ should be investigated. The finding that the large Ca^{2+} -activated K^+ channel has a very low conductance for Rb^+ should allow interesting experiments on the role of this particular channel to be carried out in various tissues.

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Revised Sm–Nd systematics of Kambalda greenstones, Western Australia

J. C. Claoué-Long*, M. F. Thirlwall† & R. W. Nesbitt*

* Department of Geology, Southampton University, Southampton SO9 5NH, UK

† Department of Earth Sciences, Leeds University, Leeds LS2 9JT, UK

Sm–Nd isotope systematics in mafic–ultramafic lavas from the Norseman–Wiluna greenstone belt, Western Australia, have been reinvestigated. A previous age based on mixing lavas and granites is not confirmed: instead, a Sm–Nd age of $3,262 \pm 44$ Myr is indicated for the lavas, 400 Myr older than intruding granites, and predating gneisses that have been proposed as basement rocks.

OPPOSING models for the development of the early crust preserved in the Yilgarn Archaean craton suggest either that gneiss terrains represent basement on which later greenstones were deposited^{1,2}, or that greenstones formed the early crust (at least in part) and granitoids were produced by partial melting of greenstones infolded to deep crustal levels³. Field relationships and geochronology are among the keys to settling this debate, but information is scarce and somewhat equivocal. Sm–Nd studies may prove useful for determining ages in the greenstone belts, but the method is hampered by a lack of preservation of original igneous minerals and the normally small range of whole-rock Sm/Nd ratios found in co-genetic suites of lavas. A previous attempt to apply Nd isotopes to the Kambalda area combined lavas with felsic intrusive rocks to generate a wide range of parent/daughter ratios, and produced an age for these samples of $2,790 \pm 30$ Myr (ref. 4). This approach is open to the danger that the samples are probably neither co-genetic nor of the same age. In acknowledging this, the previous paper notes that data for Kambalda lavas alone yield a poorly-constrained older date. Against this background, the present study discusses new Nd data for basic–ultramafic lavas in the Kambalda region.

Regional geology

Correlations within the 800 km long Norseman–Wiluna greenstone belt are complicated by extensive strike-faulting and poor exposure, but in the Kambalda area there is sufficient continuity to permit recognition of two sequences of volcanic and sedimentary rocks, referred to as the Kambalda (lower) and Bluebush (upper) sequences^{5,6} (Fig. 1). Although both horizons exhibit similar ranges of lithologies, nickel sulphides are preferentially concentrated in the Kambalda sequence. The area has been affected by at least four deformational events, as well as metamorphism (up to low amphibolite facies) and intrusion of Archaean granitoids, felsic–intermediate porphyries, minor mafic dykes and sills, and a Proterozoic dolerite dyke suite⁶. Thus all rocks are now metamorphic, but good preservation of igneous textures and chemistry merits consideration of the meta-lavas as igneous rocks.

Mafic–ultramafic lavas in the Kambalda sequence consist of >2,000 m of pillowed, or massive, fine-grained foot-wall basalts with 6–9% MgO, overlain by 0–1,000 m of ultrabasic lavas (olivine–spinel textured komatiites, liquid composition 22–31% MgO), and subsequently by >600 m of hanging-wall basalts with <15% MgO (ref. 6). These are commonly pillowed and variolitic, but can be massive or occasionally bear pyroxene–spinel. At the base of the ultrabasic formation occur one or more very thick flows, hosts to Ni–Cu sulphides, which are

layered and contain differentiation zones of basaltic pyroxene–spinel with <15% MgO. Lavas at the Kambalda Dome are intruded by a sodic granite and a series of felsic porphyries.

The Bluebush sequence is less well understood, but also consists of an ultrabasic formation sandwiched between basaltic foot-wall and hanging-wall formations. Attention here is confined to the ultrabasic formation, which is best known from good surface exposures at Ultramafic Hill and One Boob Hill, and consists of well-preserved interlayered flows of olivine–spinel textured komatiite and pyroxene–spinel textured basalt^{6,7}.

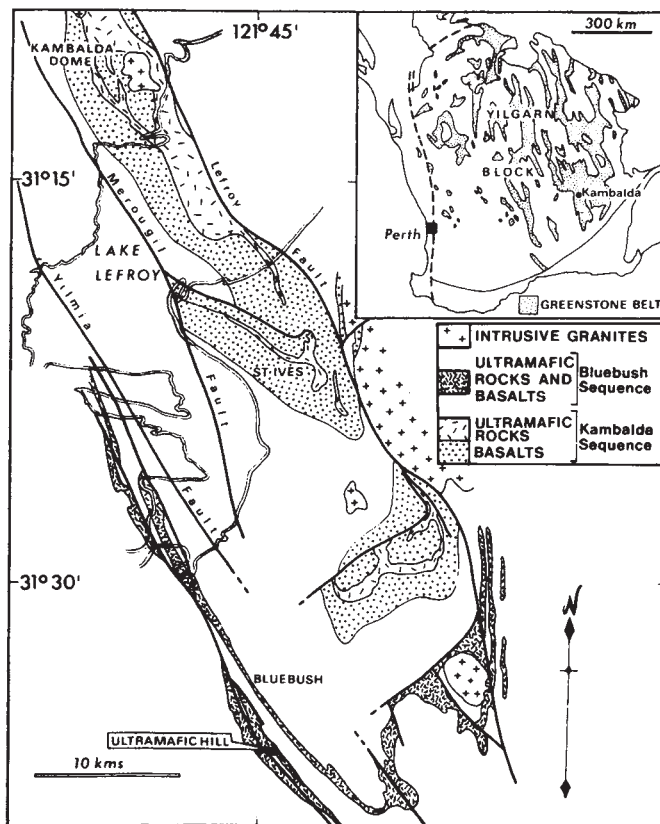


Fig. 1 Distribution of mafic and ultramafic lavas in the Kambalda–Bluebush area of the Norseman–Wiluna belt (adapted from ref. 6). Inset shows location of Kambalda in the Yilgarn block.

Table 1 Sm–Nd isotopic data

Sample	Description	Sm (p.p.m.)	Nd (p.p.m.)	$^{147}\text{Sm}/^{144}\text{Nd}$	$^{143}\text{Nd}/^{144}\text{Nd}$	$^{148}\text{Nd}/^{144}\text{Nd}$
Kambalda						
1020A/158.2	Massive, mafic HWB	2.136	8.993	0.1436	0.511610 ± 08	0.241566 ± 08
1020A/268.6	Granular, mafic HWB	1.816	6.089	0.1804	0.512413 ± 08	
1020A/473.2	Mafic HWB with acicular texture	3.069	12.203	0.1520	0.511832 ± 41	
1020A/507.3	Massive, mafic HWB	2.229	7.007	0.1923	0.512634 ± 12	0.241586 ± 10
6033/222.8	Random olivine-spinifex, thin komatiite	0.914	2.453	0.2254	0.513375 ± 14	0.241610 ± 18
6033/479.7	Random olivine-spinifex, top of thick komatiite flow	0.826	2.125	0.2352	0.513601 ± 34	
29/456.0	Random olivine-spinifex, top of thick komatiite flow	0.636	1.484	0.2592	0.514110 ± 25	
29/501.5	Pyroxene-spinifex zone, same flow as 29/456	1.164	3.462	0.2034	0.512997 ± 14	0.241587 ± 11
1025/624	Massive, mafic FWB	2.112	6.222	0.2053	0.512942 ± 22	
1029/3366	Massive, mafic FWB	0.794	2.429	0.1976	0.512762 ± 16	0.241625 ± 19
1029/4780	Massive, mafic FWB	1.982	5.986	0.2002	0.512843 ± 12	0.241600 ± 11
Bluebush						
UMH62	Devitrified glass, basalt flowtop	0.756	2.563	0.1782	0.512594 ± 32	
UMH72	Random pyroxene-spinifex, basalt	2.557	8.055	0.1920	0.512857 ± 09	
UMH74	'String beef' texture, basalt flow	2.767	8.398	0.1993	0.512943 ± 12	
UMH81	Random pyroxene-spinifex, basalt flow	2.568	7.863	0.1975	0.512879 ± 14	
UMH97	Random olivine-spinifex, komatiite	0.859	2.221	0.2340	0.513705 ± 24	0.241648 ± 22

HWB, hanging-wall basalt; FWB, foot-wall basalt. Errors quoted are 2 standard errors on the mean. $^{147}\text{Sm}/^{144}\text{Nd}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ determined on aliquots of the same sample solution. Total Nd blank is 0.2 ng and is therefore negligible for this study. $^{143}\text{Nd}/^{144}\text{Nd}$ normalized to $^{146}\text{Nd}/^{144}\text{Nd} = 0.7219$, the value determined for BCR1 = 0.512657 ± 12 . A revised calibration of the Leeds rare-earth spike using standard solutions prepared from metal ingots gives Sm/Nd = 0.3249 for the California Institute of Technology Sm/Nd Standard²⁵, and reproducibility of $^{147}\text{Sm}/^{144}\text{Nd}$ is 0.1% 2σ .

Methods and results

Much of the ensuing discussion revolves around the choice of samples. Original igneous minerals are not preserved in the Kambalda and Bluebush lavas, and whole-rocks have been used. In contrast to the previous study, which combined mafic-ultramafic volcanics with felsic plutonic rocks to produce a wide range of Sm/Nd ratios⁴, the approach adopted here is to use only mafic-ultramafic lavas. Samples from the Kambalda sequence are all from deep diamond-drill holes through the Kambalda Dome. Care was taken to choose rocks with good preservation of igneous textures, and to avoid visible forms of alteration (apart from the pervasive amphibolite facies meta-

morphism) such as biotite-rich zones, variolitic forms of basalt, and carbonation. Only surface samples are available from the Bluebush sequence: those used are all from the 400 m Ultramafic Hill section, where field relationships are best exposed, and were chosen for quality of textural preservation from portions of very large specimens free from obvious surficial weathering.

Analytical methods used for rare earth elements (REE) and Nd isotopes are described elsewhere^{8,9}. Results for Kambalda and Bluebush are listed in Tables 1 and 2, and the data are plotted on an isochron diagram in Fig. 2a. A very wide range of Sm/Nd ratios has been found in the mafic-ultramafic lavas alone, and, with one sample excluded, the new data for Kam-

Table 2 Isochron results

Data set	Age (Myr)	$(^{143}\text{Nd}/^{144}\text{Nd})^i$	ϵ_{Nd}^i	MSWD	n
Kambalda					
All lavas					
(i) New data, except 29/501.5	$3,262 \pm 44$	0.50851 ± 6	2.3 ± 0.2	3.2	10
(ii) As for (i) combined with previous data for footwall basalts & komatiites only ⁴	$3,274 \pm 57$	0.50851 ± 7	2.4 ± 0.2	5.6	14
Footwall basalts only					
(iii) New data	$3,533 \pm 1102$	0.50815 ± 148	2.2 ± 0.7	3.7	3
(iv) Combined with ref. 4	$2,930 \pm 1096$	0.50897 ± 146	2.7 ± 0.6	8.5	6
Komatiites only					
(v) New data, except 29/501.5	$3,290 \pm 140$	0.50848 ± 22	2.2 ± 0.7	0.5	3
(vi) Combined with ref. 4	$3,276 \pm 131$	0.50850 ± 20	2.3 ± 0.7	0.4	4
Hanging-wall basalts only					
(vii) New data	$3,226 \pm 136$	0.50855 ± 15	2.1 ± 0.6	8.6	4
(HWB data in ref. 4 omitted, see discussion)					
Bluebush					
All lavas	$3,045 \pm 554$	0.50896 ± 73	5.4 ± 0.9	37.0	5

Regressions by the method of York²⁶. All errors are 2σ , and are multiplied by $\sqrt{\text{MSWD}}$ when $\text{MSWD} > 1$. ϵ_{Nd}^i is a measure of the deviation in $^{143}\text{Nd}/^{144}\text{Nd}$ of a suite of samples from a reference composition CHUR (chondritic uniform reservoir)²⁷, and is calculated by comparing the present day composition, $(^{143}\text{Nd}/^{144}\text{Nd})_{\text{Sa}}^0$, of a hypothetical sample having $(^{147}\text{Sm}/^{144}\text{Nd})_{\text{Sa}}^0 = (^{147}\text{Sm}/^{144}\text{Nd})_{\text{CHUR}}^0$, with the present day composition, $(^{143}\text{Nd}/^{144}\text{Nd})_{\text{CHUR}}^0$, of CHUR according to the definition of ref. 21:

$$\epsilon_{\text{Nd}}^i = \left[\frac{(^{143}\text{Nd}/^{144}\text{Nd})_{\text{Sa}}^0}{(^{143}\text{Nd}/^{144}\text{Nd})_{\text{CHUR}}^0} - 1 \right] \times 10^4.$$

Present day reference values for CHUR²⁸ are:

$$(^{143}\text{Nd}/^{144}\text{Nd})_{\text{CHUR}}^0 = 0.51264 \quad \text{and} \quad (^{147}\text{Sm}/^{144}\text{Nd})_{\text{CHUR}}^0 = 0.1967.$$

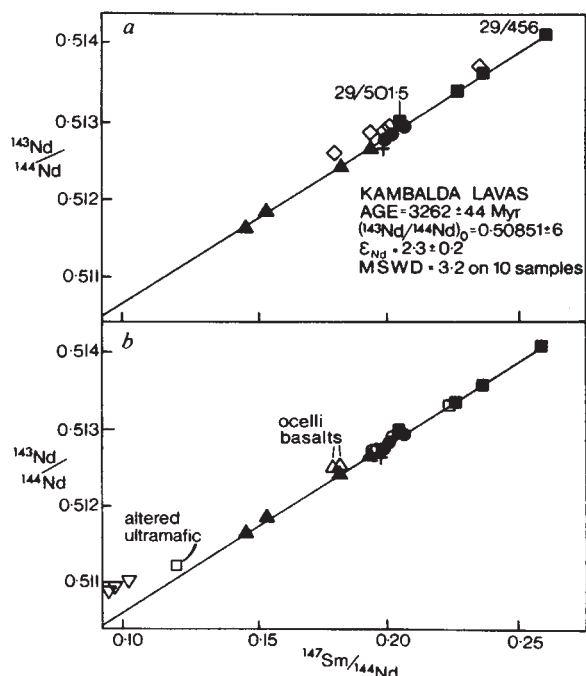


Fig. 2 *a*, Sm-Nd evolution diagram for Kambalda and Bluebush data in Table 1. Isochron plotted is for Kambalda only (omitting sample 29/501.5, see discussion). *b*, Data and isochron for Kambalda plotted as in *a* (solid symbols) compared with data from ref. 4 (open symbols). Previous data for ocelli-basalts do not coincide with purely mafic lavas in the hanging-wall formation (new data), and the tightly-constrained lavas isochron does not extrapolate to points representing intrusive granites. Kambalda: ●, footwall basalts; ■, komatiites; ▲, hanging-wall basalts; ▽, granites. ◇, Bluebush lavas. + Represents the present-day chondritic value.

balda yield a tightly constrained isochron (MSWD = 3.2) of age $3,262 \pm 44$ Myr with an initial $^{143}\text{Nd}/^{144}\text{Nd}$ of 0.50851 ± 6 and $\epsilon_{\text{Nd}} = 2.3 \pm 0.2$. Data for Bluebush do not form an isochron, but plot separately from the Kambalda line and clearly have a much higher ϵ_{Nd} value in the region of 5–6.

The sample excluded (29/501.5) from the otherwise coherent new data set for Kambalda is from the basaltic differentiation zone of a layered komatiite flow whose flowtop is also analysed (sample 29/456, see Fig. 2a). It is not possible to ascribe the severe divergence in Sm/Nd ratio (22%) between this sample and the flowtop composition to simple crystal fractionation within the flow, and it is unique in departing from the otherwise regular and predictable variation found in the rest of the data set. 29/501.5 lies close to a minor basic intrusion, and its unusual Sm-Nd composition suggests that a process of exchange of light-REE (LREE) with the younger intrusion may have occurred.

The new results for the Kambalda lavas are not colinear on the isochron diagram with re-normalized data from a previous study⁴ (Fig. 2b). Unfortunately there are no sample descriptions in the previous paper, but the two basalts most divergent from the new data are referred to as ocelli-bearing. This type has been avoided in the present study in the belief that purely mafic varieties are more likely to preserve original igneous geochemistry. An anomalous ultramafic sample is also reported by the previous authors, who believe it unlikely to represent original igneous geochemistry (for reasons similar to those given above with respect to sample 29/501.5), and they report that it came from an alteration zone at the margin of a felsic porphyry.

A total of 18 samples of lavas were selected by this and the previous study as *a priori* candidates for analysis, and definition of an isochron. Of these, it is apparent that ocelli-basalts and an altered ultramafic rock from the previous paper, and a further ultramafic sample from this study, no longer possess original

igneous Sm-Nd compositions, and they are excluded from further discussion. Rejection of these samples does not invalidate the regression defined by the remaining lavas, but does make it less easy to justify. However, it is notable that the four other data points for lavas used in the previous study (one ultramafic, three foot-wall basalts) plot together with the new data. Combining these four data points with the new analyses yields a line within error of that based on the new results alone, and a comparable age ($3,274 \pm 57$ Myr), initial $^{143}\text{Nd}/^{144}\text{Nd}$ (0.50851 ± 7), and ϵ_{Nd} (2.4 ± 0.2).

The poor coherence of data for the Bluebush lavas is most unlikely to reflect variation in ages of the flows that were sampled. The scatter could represent original variations in initial $^{143}\text{Nd}/^{144}\text{Nd}$, but it is not possible to rule out disturbance of the Sm-Nd isotopic system by weathering of the surface samples.

Whole-rock 'isochrons'

To obtain a wide spread of parent/daughter ratios, the previous Sm-Nd study of Kambalda enlisted a mixture of whole-rock samples ranging from komatiite to granite, on the assumption that such diverse samples may have the same age and essentially homogeneous initial $^{143}\text{Nd}/^{144}\text{Nd}$ (ref. 4). This assumption is the main weakness of whole-rock dating applied in the absence of mineral data. Justification for such studies can come from the internal consistency of results so obtained, and indeed the previous interpretation of the komatiite-granite correlation as a real age for Kambalda was based on the tightness of the regression through all the samples used. However, the slope of this regression was largely controlled by the samples of granite, and the large variation in Sm/Nd now found in the mafic-ultramafic lavas alone produces a tightly-constrained line that does not extrapolate through the granite data (Fig. 2b). This strongly suggests that the lavas and granites were derived from isotopically distinct sources, and that the age derived from mixing the plutonic and volcanic samples is spurious. However, it remains to be shown that the correlation within the lavas is not a mixing line between magmas from an isotopically heterogeneous mantle.

Reference to Fig. 3 shows that, regardless of Sm/Nd ratios, all lavas in the Kambalda sequence have essentially flat heavy-REEs (HREEs), whereas Bluebush lavas show HREE depletion. This difference corresponds to a worldwide division of komatiite types previously thought to reflect temporal changes in komatiite genesis¹⁰: early HREE-depleted komatiites (for example, in the Barberton belt) are thought to result from garnet control in the mantle, whereas younger lavas so far studied have chondritic ratios of HREE and do not show the influence of garnet separation. The temporal sequence of source regions is reversed with the Kambalda and Bluebush lavas, and their close spatial association alone is evidence that mantle heterogeneity existed below the Kambalda region during the Archaean.

The foot-wall, komatiite and hanging-wall formations at Kambalda each represent co-magmatic lava batches. Within each formation, variations in Sm/Nd ratio (34% for the hanging-wall basalts, 27% for the komatiites) may be ascribed to either the combined effects of differing degrees of partial melting of, and fractionation in, magmas from a homogeneous source, or the possibility that Sm/Nd in each lava directly reflects variations in Sm/Nd within the source area. Continuity of source composition between formations is not easy to justify because the variation in Sm/Nd ratio between lava batches is large (Fig. 3), and a very high degree of LREE fractionation is indicated if the same source is involved.

Taking the worst case, it is possible that the correlation on the isochron diagram is a mixing line between lavas derived from two, perhaps more, mantle sources possessing different Sm/Nd ratios. This leaves two main alternatives: (1) the lavas were erupted 3,200–3,300 Myr ago as the lavas correlation suggests. This requires that any Sm/Nd heterogeneity between the two (or more) mantle sources existed for a very short time before eruption of the three batches of magma. (2) The lavas

were erupted at 2,700–2,900 Myr as has previously been supposed. In this case the correlation in Fig. 2 represents mixing of data from a mantle that possessed long-lived heterogeneities in Sm/Nd.

These alternatives may be investigated by examining how data points for the individual lava batches fit the isochron. If lavas are co-genetic within each of the Kambalda formations, then individual true isochrons are defined by the limited numbers of points in each group. Errors on the ages yielded by these domains within the isochron are large, because of the limited spread of Sm/Nd ratios found in any one formation, but they are equal within error and constrain the age to older than 3,100 Myr (see Table 2). An additional constraint arises from the fact that the age deduced from the whole data set lies within the errors of the ages deduced from the individual formations. If mantle heterogeneity in Sm/Nd had existed for a significant time before eruption, then each source would have evolved $^{143}\text{Nd}/^{144}\text{Nd}$ separately to generate distinct initial ratios. As a consequence, isochrons through the domains would be parallel, but offset relative to one another by an amount proportional to the degree, and duration, of the heterogeneity. The regression

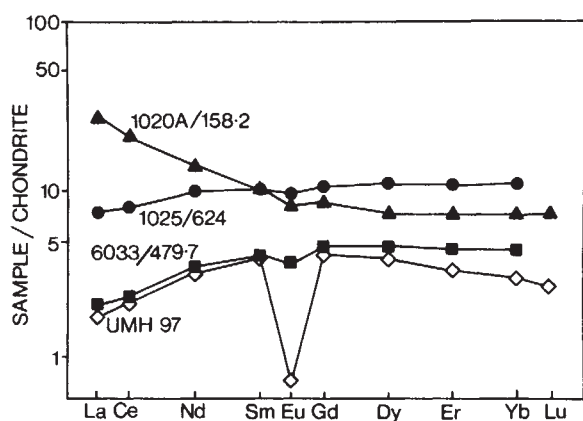


Fig. 3 Chondrite normalized REE abundances for selected Kambalda and Bluebush samples (symbols as for Fig. 2). Kambalda lavas have essentially flat HREE patterns, and show pronounced variation in LREE slopes. The Bluebush komatiite has identical LREE depletion to its Kambalda counterpart, but is depleted in HREE also. The large negative Eu anomaly may be due to alteration in the Bluebush surface sample.

through all the data would give an older age than isochrons through the individual magma batches. This 'tram line' effect is not observed in the Kambalda data, a point which is also illustrated by the homogeneous ϵ_{Nd} of the subsets.

Another explanation of Sm–Nd variations in the Kambalda samples is that the Sm/Nd ratio of each lava directly reflects Sm/Nd heterogeneities within the source region. This implies that essentially no fractionation of LREE occurred during partial melting and eruption and, therefore, the $3,262 \pm 44$ Myr age given by the lavas isochron would refer to the date of differentiation of sources within the mantle, not necessarily the date of eruption. If the lavas were subsequently erupted at 2,800 Myr, this model requires that the hanging-wall basalts were partially melted from a source which had been LREE-enriched for 400 Myr. Thus sample 1020A/158.2 would have to come from mantle source with $\epsilon_{\text{Nd}} = -1.0$ at 2,790 Myr. However, available evidence suggests that the Archaean mantle was characterized by long-lived depletion of large-ion lithophile (LIL) elements^{11–13} and it seems probable that if a LREE-enriched region existed in the mantle below Kambalda, it was created a short time before, or during, the partial melting event.

These arguments suggest that, while variations in Sm/Nd ratio almost certainly existed in the source of the Kambalda lavas, they were short-lived. Any heterogeneity of initial $^{143}\text{Nd}/^{144}\text{Nd}$ was therefore insignificant in terms of the error of the Nd age, and it is concluded that the data yield a true age for the lavas, which were erupted $>3,200$ Myr ago.

Archaean mantle

In the compositions of the Kambalda and Bluebush lavas there lies evidence for the existence of a wide variety of source regions. The revised Kambalda ϵ_{Nd} value of 2.3 ± 0.2 is 30% lower than that obtained from the komatiite–granite regression⁴. However, it confirms the depleted source for the greenstones found by the previous authors, is consistent with ϵ_{Nd} values obtained for Archaean mafic–ultramafic volcanics of various ages in other cratons^{11–13}, and is further evidence for worldwide distribution of long-lived LIL-element depletion in at least part of the Archaean mantle. It is argued above that this already-depleted mantle may have undergone a separation, shortly before 3,200–3,300 Myr ago, into compositions ranging from LREE depleted to LREE enriched, and within a short time span these became sources for the three batches of magma erupted at Kambalda.

Another window into the mantle is provided by the stratigraphically-younger Bluebush sequence. Despite the scatter of data for these lavas, it is apparent that they derive from a very radiogenic source ($\epsilon_{\text{Nd}} = 5.4 \pm 0.9$). The high ϵ_{Nd} may be an artefact of systematic alteration of Sm–Nd isotopes in the surface samples, but it corresponds with differences in HREE (Fig. 3), which suggest a garnet residue in the Bluebush source but not in the Kambalda source(s). It is most unlikely that the source of even the most depleted samples from the Kambalda sequence could have evolved ^{143}Nd sufficiently rapidly to be the source for Bluebush also, and it is inferred that yet another mantle composition was available at the time that the Bluebush lavas were erupted.

The Kambalda granite

The sodic granite used in the previously-published Sm–Nd isochron for Kambalda has been independently dated at $2,820 \pm 15$ Myr by U–Pb on zircons¹⁴. To produce a Sm–Nd isochron by combining this granite with the mafic–ultramafic lavas it intrudes requires the assumption that the felsic samples have the same initial $^{143}\text{Nd}/^{144}\text{Nd}$ as, and are essentially the same age as, the mafic–ultramafic lavas⁴. However, it is most unlikely that the granite is directly derived from the mantle source of the lavas, and an origin by extreme fractional crystallization from the lavas is also highly unlikely because of the large LREE fractionation required. Thus it is not surprising that the new Sm–Nd isochron for Kambalda, based on lavas alone, does not extrapolate through the granite data (Fig. 2b).

Other possible origins for the Kambalda granite are that it represents re-melting of old granite–gneiss basement, or that it derives from a partial melt of older greenstone rocks infolded to great depths. It is possible to choose between these options because the ϵ_{Nd} of the granite is independently set at 3.9 ± 0.6 using the zircon age^{4,14}. The high ϵ_{Nd} raises the paradox that the granite (which is very LREE enriched⁴) was derived from LREE depleted (high Sm/Nd) source, and this does not correspond to known compositions of granite–gneisses in the area¹⁵. This leaves the possibility that the 2,800 Myr-old granite was melted from pre-existing greenstones. Suitably light-REE enriched liquids would result if such material, in the form of garnet–amphibolite or garnet–granulite, was partially melted at depth⁴, and basic–ultrabasic lavas comprising the bulk of this, and other, greenstone belts do have the radiogenic Nd compositions required to form a source for the granite^{11–13}. For instance, it could be produced by melting of Kambalda greenstones with average $^{147}\text{Sm}/^{144}\text{Nd} = 0.2262$, which is close to the mean measured value at Kambalda. This is independent, if indirect, evidence that greenstones much older than the 2,800 Myr-old granite exist in the Yilgarn block.

Crustal evolution

Detailed geochronological studies in the Yilgarn block are sparse, but reported zircon ages of $2,560 \pm 30$ Myr for some minor felsic dykes, and $2,820 \pm 15$ Myr for the granite, both at Kambalda¹⁴, are significantly younger than the $3,262 \pm 44$ Myr-old lavas that they intrude. It has been suggested that

the felsic intrusives are a subvolcanic expression of felsic volcanism higher in the greenstone stratigraphy¹⁶, and a whole-rock Sm-Nd date of $2,780 \pm 70$ Myr has been reported for felsic volcanics at Kanowna, 60 km north of Kambalda¹³. If all these ages are correct, this implies that development of the greenstone belt extended over a period in excess of 380 Myr and highlights a remarkable lack of recognized unconformities—a paradox that applies to many, if not most, greenstone belts¹⁷.

The regionally extensive banded gneisses, which have been considered as representing the basement to the greenstones¹⁸, have yielded a whole-rock Sm-Nd age of $2,800 \pm 100$ Myr for localities close to Kambalda¹⁵, and a whole-rock Pb-Pb age of $2,700 \pm 97$ Myr for samples from widespread localities in the Yilgarn granite-greenstone terrain¹⁹. These are younger than both the age reported here for the Kambalda greenstones, and a further whole-rock Sm-Nd age of $3,050 \pm 100$ Myr reported for greenstones in the Diemals-Marda area¹³. This observation does not correspond with simpler models of craton development whereby greenstones are late features deposited on granite-gneiss basement.

Older dates are found in gneisses of the Western Gneiss Terrain, from where zircon dates of $3,267^{+48}_{-25}$ Myr for Gabidine Springs and $3,341^{+133}_{-73}$ Myr for Windmill Hill, comparable in age to the Kambalda volcanics, have been reported²⁰. Attention has recently focused on gneisses near Mount Narryer which have produced whole-rock Rb-Sr and Sm-Nd ages of $3,300$ – $3,600$ Myr²¹, and in which have been found four detrital zircons yielding near-concordant U-Pb ages between 4,100 and 4,200 Myr (refs 22, 23). Isotopic dates in the Yilgarn block thus cover a period of over 1,500 Myr. Together with the great age of the Kambalda greenstones in relation to associated banded gneisses, this suggests that formation of the early crust in the Yilgarn was a multi-stage process, and more complex models must be sought.

Conclusions

Sm-Nd systematics in rocks from the Kambalda region of the Yilgarn Craton, Western Australia, yield important constraints on the evolution of the Archaean mantle and crust. The paradox of low Sm/Nd but high ϵ_{Nd}^1 in a 2,800 Myr-old granite suggests that it represents a partial melt of older greenstone material rather than granitic gneisses. This indirect clue to the existence

of very old greenstones in the Yilgarn is confirmed by the $3,262 \pm 44$ Myr Sm-Nd age of lavas hosting the Kambalda nickel deposits, which predate gneisses previously proposed as basement rocks. The known development of this section of the Norseman-Wiluna belt is extended to a period of more than 380 Myr.

The lavas at Kambalda may have been derived from at least two sources, with distinct Sm/Nd ratios, that separated from a mantle with $\epsilon_{Nd}^1 = 2.3 \pm 0.2$ immediately before partial melting and eruption. Scatter of data in volcanics from an overlying sequence at Bluebush may reflect disturbance of the Sm-Nd system. However, ϵ_{Nd}^1 is systematically very high for all samples from Ultramafic Hill, and this combines with other characteristics to suggest that a separate part of the mantle was tapped to generate the younger volcanics. A previously-suggested evolution of komatiites with time, from HREE-depleted types to lavas with chondritic ratios of these elements is not confirmed, and it is inferred that a wide variety of mantle compositions was available under the Kambalda region during development of the greenstone belt.

That the Kambalda lavas are apparently 400 Myr older than previous dates from the same greenstone belt²⁴ is perhaps an artefact of the severe lack of Sm-Nd studies in the area, and the fact that existing chronologies depend on decay schemes such as Rb-Sr, K-Ar and U-Th-Pb, which either have not recorded primary crystallization ages or have only been applied to younger intrusive rocks. However, this study shows that confidence placed in whole-rock isochrons is clouded by little-discussed problems of source heterogeneity and choice of samples. Further detailed applications of Sm-Nd isotopes are required to substantiate this presently anomalous result.

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Alteration of *c-myc* chromatin structure by avian leukemia virus integration

William Schubach* & Mark Groudine†

Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98014, USA

† Department of Radiation Oncology, University of Washington Hospital, Seattle, Washington 98015, USA

The most common sites of integration of the leukemia virus (ALV) long terminal repeat (LTR) in bursal lymphomas and derivative cell lines correspond to a region encompassed by two major hypersensitive sites in the 5' flanking region of the pre-integration, unrearranged c-myc gene. After integration of the ALV LTR, the major hypersensitive site within the avian c-myc oncogene region is within the proviral LTR, and the major hypersensitive sites normally found in uninfected cells 5' to the first c-myc coding exon are no longer detectable.

DIFFERENTIAL sensitivity to DNase I distinguishes the chromatin structure of genes that are actively transcribed from those that are inactive¹. In addition, specific DNase I hypersensitive sites have been found in a variety of systems, predominantly near the 5' ends of actively transcribed genes (refs 2–8; see also ref. 9 for a review). In some cases, these sites appear with the developmentally regulated transcriptional activation of specific genes^{4–6}, and in other systems, their appearance precedes the activation of gene expression^{7,8}. Hypersensitive sites have also been detected within certain regulatory sequences in DNA, such as the long terminal repeats (LTRs) of transcribed avian endogenous provirus^{10,11}, the 72 base pair (bp) repeat involved in enhancement of simian virus 40 (SV40) gene expression^{12,13}, and an enhancer complex located upstream from an actively transcribed glue protein gene in *Drosophila*^{14,15}. It is possible that the local alteration in chromatin structure revealed by DNase I hypersensitivity is related to the apparent enhancer function of these elements.

There is also indirect evidence that DNase I hypersensitivity characterizes regions involved in targeted recombination. In yeast mating-type interconversion, a specific and genetically regulated DNase I hypersensitive site corresponds to the site of a double-strand endonucleolytic cleavage that is destined to be the recipient site in the active cassette¹⁶. Also, in T lymphocytes, a cluster of DNase I hypersensitive sites occurs within a span of several kilobases between joining (*J*) and heavy chain (*C_H*) genes in the region involved in heavy chain switching in B lymphocytes¹⁷.

To understand the possible relationships among hypersensitive sites, 'enhancement' and recombination, we studied the DNase I hypersensitivity pattern upstream from the cellular oncogene of chickens, *c-myc*. *c-myc* is the endogenous cellular homologue of the transforming gene (*v-myc*) of the avian myelocytomatosis virus, MC29^{18–20}. In bursal lymphomas induced by the avian leukemia virus (ALV), integration of the ALV provirus in the region of *c-myc* has been shown to enhance significantly transcription of the *c-myc* gene²¹. In addition, rearrangements around *c-myc* in B-cell tumours of other species have been described. For example, in mouse myeloma^{22,23} and in Burkitt's lymphoma^{24–27} cell lines translocations involving immunoglobulin genes and *c-myc* are associated with altered *c-myc* transcription. Thus, we felt a study of the chromatin structure around the *c-myc* oncogene prior to and after LTR integration into this locus might provide insight into amplified or altered gene expression in bursal lymphomas. This was facilitated by the isolation of bursal lymphoma cell lines haploid for *c-myc*, containing only a single *c-myc* allele associated with an upstream ALV proviral LTR. Thus, the chromatin structure of

the normal, unrearranged *c-myc* locus does not interfere with the analysis of the structure of the LTR-associated *c-myc* chromosomal domain. Our results show that after the integration of the ALV LTR, the major hypersensitive site within the *c-myc* region usually occurs within the proviral LTR, and the major hypersensitive sites normally found in uninfected cells 5' to the first *c-myc* exon are no longer detectable. Also, we find that the most common sites of integration of the ALV LTR in bursal lymphomas and derivative cell lines correspond to a region encompassed by two major hypersensitive sites in the 5' flanking region of the unrearranged *c-myc* gene.

Mapping hypersensitive sites

The presence and location of hypersensitive sites in the *c-myc* locus were determined by treating nuclei of various tissues obtained from embryonic and young chicks with increasing concentrations of DNase I for brief periods of time. DNA isolated from these nuclei was subsequently digested with restriction endonuclease, electrophoresed on agarose gels and blot hybridized to nick-translated probes derived from a genomic clone of the *c-myc* region. In this analysis, hypersensitive sites are manifest as DNase I generated sub-bands, one end of which is defined by the restriction enzyme recognition site and the other by the DNase I hypersensitive site.

Figure 1 shows the results of this type of analysis of nuclei derived from 14-day embryonic red blood cells (RBC) and a bursa from a 4-week post-hatching chicken. In Fig. 1a, RBC nuclei were digested with DNase I, and the DNA was isolated and digested with *Sst*I, 'Southern' blotted and probed with the 9.2-kilobase (kb) λ -*myc* probe depicted at the bottom of the figure. This probe includes the coding and 5' flanking sequences of the *c-myc* region. In addition to the 7.3-kb *Sst*I parent fragment, two additional bands of 4.8 and 5.8 kb are generated by DNase I. Extensive mapping of these sub-bands has revealed two hypersensitive sites (designated II and III in Fig. 1a) upstream from *c-myc*. The 3.2-kb *Sst*I band, which encompasses the *c-myc* coding region, is very sensitive to digestion by DNase I since it is smaller and disappears before the sub-bands.

Because *Sst*I cleaves 190 bp downstream from the identified 5' coding sequences of *c-myc*²⁸, it is not a suitable restriction enzyme for defining hypersensitive sites close to the 5' end of this coding region. Thus, we used the indirect end-labelling approach of Wu², in which a probe is used that hybridizes to that end of the sub-band defined by the restriction endonuclease recognition site. With this approach, the location of DNase I hypersensitive sites can be inferred directly from the length of the sub-bands generated. Nuclei from a 4-week bursa were digested with increasing concentrations of DNase I, restricted with *Cla*I and *Bam*HI, Southern blotted, and hybridized to a 1,050-bp probe the 3' end of which is defined by the *Cla*I site and which spans the *c-myc* intron indicated in the map below

* Present address: Department of Medicine, University of Minnesota School of Medicine, Minneapolis, Minnesota 55455, USA.

Fig. 1 Endogenous DNase I hypersensitive sites upstream from *c-myc*. Nuclei from designated tissues were digested with increasing concentrations of DNase I (as designated by the direction of the arrow), DNA isolated, digested with the enzymes denoted, and subjected to blot hybridization following electrophoresis in 1% agarose gels. The blots were probed with the nick-translated probes designated below the autoradiograms. Nuclear isolation, DNase I digestion of nuclei, DNA purification, restriction enzyme digestion and blot hybridization were performed as described previously^{4,5,8}. All probes described in this and subsequent figures containing *c-myc* sequences were derived from a λ clone containing a 9.2-kb insert with sequence homology to *v-myc*, isolated from a chicken genomic library⁴⁷ by conventional screening methods⁴⁸. Plasmid subclones were prepared using the pBR322 analogue pSV2 (obtained from B. Seed). Published data^{28,30,49} have been used to correlate specific fragments with intron and exon positions. λ -*myc* is a 9.2-kb *EcoRI* fragment with an artificial R1 site at the 5' end and a 3' end that is the *EcoRI* site at the 3' side of the second *c-myc* exon. 'Intron' is a 1-kb *Sall*-*ClaI* fragment that includes primarily the intron of *c-myc*. Fragments were isolated from their host-vector sequences by restriction enzyme digestion, agarose gel electrophoresis, isolation in hydroxyapatite troughs⁵⁰ and nick-translated⁵¹. Restriction sites are marked; E, *EcoRI*. *a*, Four week RBC were digested with *SstI* and hybridized to the λ -*myc* probe. A 3-kb *SstI* band detected by this probe is very sensitive to DNase I. A parent band of 7.3 kb and sub-bands of 5.8 and 4.8 kb are seen corresponding to the hypersensitive sites III and II in the diagram at the bottom of the figure. *b*, Normal 4-week bursa DNA digested with *ClaI* plus *BamHI* and analysed using the 1-kb intron probe depicted at the bottom of the figure. A parent fragment of 5.8 kb and sub-bands corresponding to the arrows are seen. See text for details.

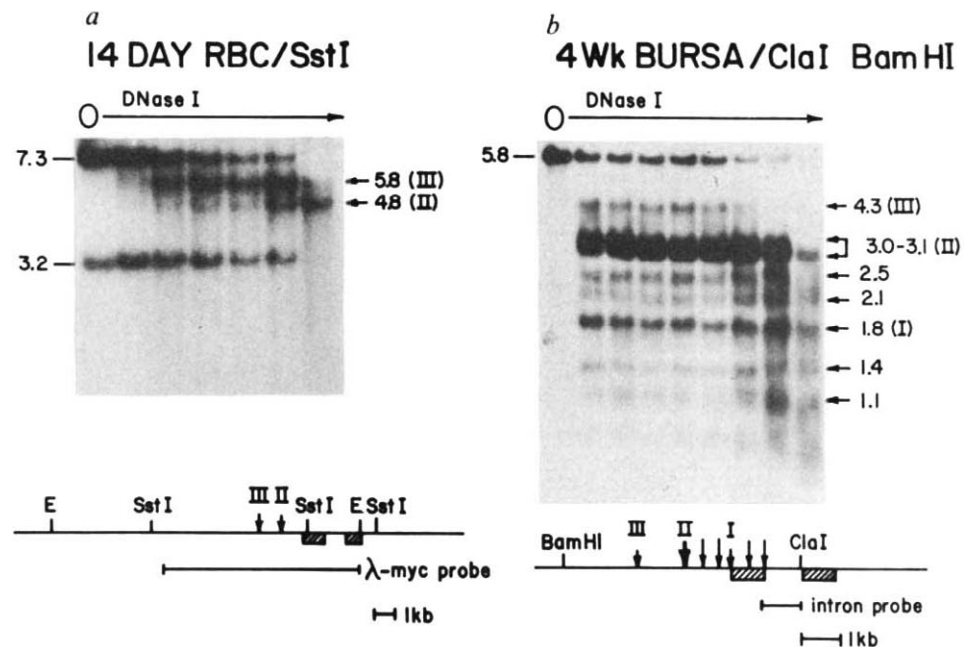


Fig. 1b. Figure 1b shows that *ClaI*/*BamHI* digestion of normal bursa DNA reveals a parent band of 5.8 kb and DNase I-generated sub-bands of 4.3, 3.0–3.1, 2.5, 2.1, 1.8, 1.4 and 1.1 kb; arrows show positions of the specific DNase I hypersensitive sites in *c-myc* from which these sub-bands are generated. Since the *ClaI* site is 1.8 kb from the 5' side of the first *c-myc* exon²⁸, and the 3' end of the probe is defined by this *ClaI* site, we can determine fairly accurately the positions of these hypersensitive sites relative to the 5' side of the first *c-myc* exon. A prominent hypersensitive site lies 1.2–1.3 kb upstream from the first exon (site II). Less prominent, but distinct sub-bands lie at the end of the first exon (site I) and 2.5 kb upstream from the first exon (site III). Minor DNase I hypersensitive sites lying between I and II, at the 3' end of the first exon, and within the intron are also designated in Fig. 2b by arrows. These latter sites vary in intensity and appearance in different nuclear preparations.

Results comparable with those in Fig. 2b were obtained for 14-day embryonic RBC and yolk sac cells, and bursa, thymus, liver, brain, bone marrow, muscle and spleen from a 4-week-old chick, as well as cultured chick embryonic fibroblasts (CEF), Rous sarcoma virus (RSV)-transformed CEF and MSB cells²⁹. In addition, when the same blots are probed with sequences from a gene known to be inactive in these tissues (for example, a probe for sequences flanking the endogenous retroviral locus *ev-1*), no hypersensitive sites are seen, and the *ev-1*-containing sequences are less sensitive to DNase I than *c-myc* coding sequences (data not shown). Thus three prominent DNase I hypersensitive sites, denoted I, II and III, lie upstream from *c-myc* in the chromatin from the majority of chicken tissues studied at these stages of development, and the *c-myc* sequences appear to be in an active chromatin structure in all of these cells.

Cell lines: restriction mapping

We have analysed the restriction sites and mapped the location and orientation of the LTR for five bursal lymphoma cell lines. The data and results of this analysis are presented in Fig. 2, and for comparison the restriction map of the *c-myc* region of normal chicken DNA³⁰ is included in Fig. 2b.

Figure 2a shows the analysis of DNA from a diploid bursal lymphoma cell line, BK3A. When DNA from these cells is digested with *EcoRI* and probed with the *c-myc* probe (shown at the bottom of Fig. 2b), three bands are generated: the 14- and 20-kb bands correspond to fragments that flank the *EcoRI* site in the 'normal' *c-myc* allele. A third 3.7-kb fragment is generated by the introduction in an ALV LTR upstream from *c-myc* due to the presence of an *EcoRI* site in the U3 region of the LTR³¹. Since this band is detected by a probe for sequences of the U5 region of the LTR, but not by a probe for U3 sequences (Fig. 2a), this 3.7-kb tumour-specific junction fragment demonstrates that an LTR is located 1.0 kb upstream from the first major *c-myc* exon in the same transcriptional orientation as *c-myc*. This arrangement has been confirmed by digestion with *BamHI*, *SstI* and *Sall* and probing with defined segments of *c-myc* and the ALV genome (not shown). Double digestion of BK3A with *BamHI* plus *Sall* and probing with the 5' exon of *c-myc* confirms the arrangement of LTR and *c-myc* sequences in BK3A cells (Fig. 2b).

The same analyses were performed for the biologically cloned cell lines BK25 and 1104HI, and these results are illustrated in Fig. 2a. In both cases digestion with *EcoRI* generates only one band that is detected by probes for both *c-myc* and 5' LTR sequences. The higher molecular weight *myc* bands are not seen, and *BamHI*/*Sall* double digestion detects only one band containing 5' exon sequences. Thus, these cells contain only one *c-myc* allele, the one associated with a nearby LTR, the 'normal' *c-myc* allele having been lost. In BK25 cells, the LTR lies within 0.1–0.2 kb of the first *c-myc* exon, and in 1104HI the LTR lies 1.2 kb upstream from the first *c-myc* exon. In both cases, the LTR is in the same transcriptional orientation as *c-myc*, as depicted in Fig. 2b.

A similar analysis of a subclone from the 1104B1 cell line, 1104BI S13, is depicted in Fig. 2a. In this case, *EcoRI* yields a single band of 5.4 kb that hybridizes to *c-myc* and 3' LTR, but not 5' LTR probes. Thus, in this cell line, the LTR and contiguous *c-myc* sequences lie in opposite transcriptional orientations, and only the LTR-associated *c-myc* locus is present.

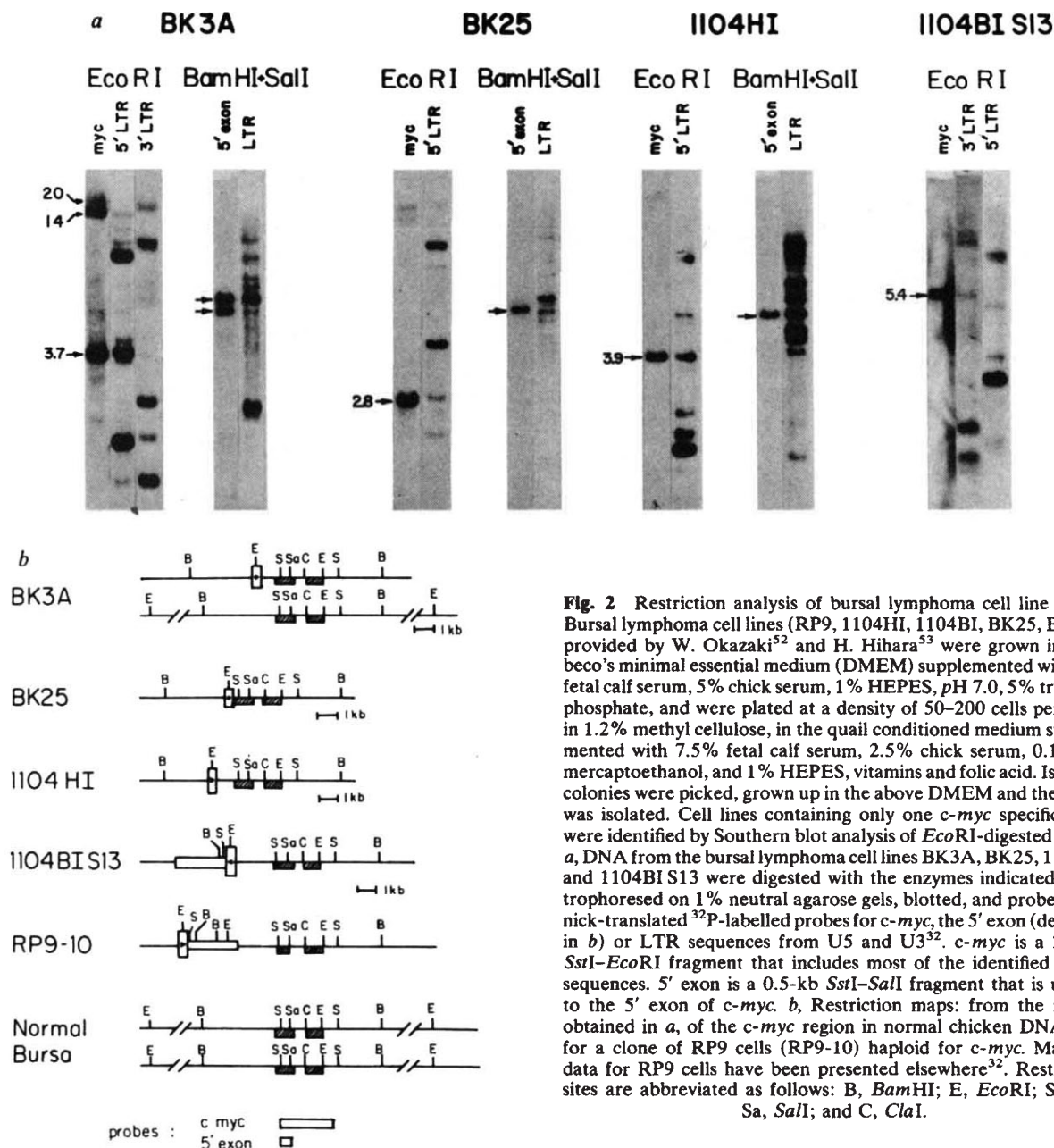


Fig. 2 Restriction analysis of bursal lymphoma cell line DNA. Bursal lymphoma cell lines (RP9, 1104HI, 1104BI, BK25, BK3A) provided by W. Okazaki⁵² and H. Hihara⁵³ were grown in Dulbecco's minimal essential medium (DMEM) supplemented with 5% fetal calf serum, 5% chick serum, 1% HEPES, pH 7.0, 5% tryptose phosphate, and were plated at a density of 50–200 cells per plate in 1.2% methyl cellulose, in the quail conditioned medium supplemented with 7.5% fetal calf serum, 2.5% chick serum, 0.15 mM mercaptoethanol, and 1% HEPES, vitamins and folic acid. Isolated colonies were picked, grown up in the above DMEM and the DNA was isolated. Cell lines containing only one *c-myc* specific band were identified by Southern blot analysis of *EcoRI*-digested DNA. **a**, DNA from the bursal lymphoma cell lines BK3A, BK25, 1104HI and 1104BI SI3 were digested with the enzymes indicated, electrophoresed on 1% neutral agarose gels, blotted, and probed with nick-translated ³²P-labelled probes for *c-myc*, the 5' exon (depicted in **b**) or LTR sequences from U5 and U3³². *c-myc* is a 2.4-kb *SstI*-*EcoRI* fragment that includes most of the identified *c-myc* sequences. 5' exon is a 0.5-kb *SstI*-*SalI* fragment that is unique to the 5' exon of *c-myc*. **b**, Restriction maps: from the results obtained in **a**, of the *c-myc* region in normal chicken DNA, and for a clone of RP9 cells (RP9-10) haploid for *c-myc*. Mapping data for RP9 cells have been presented elsewhere³². Restriction sites are abbreviated as follows: B, *BamHI*; E, *EcoRI*; S, *SstI*, Sa, *SalI*; and C, *ClaI*.

Preliminary restriction analysis (in collaboration with M. Linial) of the *myc* region in this subclone shows that there are approximately 2.5 kb of proviral sequences inserted 2.6–2.7 kb upstream from the first *c-myc* exon (Fig. 2b).

When RP-9 cells are biologically cloned, clones containing only the *c-myc* allele and upstream proviral sequences are obtained. The restriction map of one such clone, RP9-10, is shown in Fig. 2b. Detailed mapping of *c-myc* in RP-9 cells has been presented elsewhere³². Essentially, this clone contains approximately 2.5 kb of proviral information consisting of a complete LTR and *gag* gene and part of *pol* gene, inserted 2.5–2.6 kb upstream from the first *c-myc* exon. The proviral sequences and *c-myc* are in the same transcriptional orientation (Fig. 2b).

Cell lines: chromatin structure

In most bursal lymphomas induced by ALV, the proviral LTR is integrated upstream from *c-myc* such that regulatory sequences in the LTR act directly to promote or otherwise enhance transcription of *c-myc*^{21,33–35}. In addition to these types of integrations which are consistent with a downstream promoter model for the LTR activation of *c-myc*²¹, enhanced *c-myc*

expression has been reported in bursal lymphomas in which the LTR is located downstream from *c-myc* or upstream from *c-myc* in the opposite transcriptional orientation³⁶. Since retroviral LTRs or analogous elements have been found to have an enhancer function distinct from that associated with downstream promotion^{37–39}, and because DNase I hypersensitive sites are associated with enhancer sequences in SV40 chromatin^{12,13} and the *Drosophila Sgs4* gene¹⁴, we asked whether the introduction of an LTR upstream from *c-myc* altered the pattern of DNase I sensitivity that we observed in uninfected tissues. Such an analysis is difficult in tumours where *c-myc* is diploid. One locus contains a nearby LTR and one does not. Thus, there is both a 'normal' allele containing the family of endogenous hypersensitive sites, and an 'activated' allele containing an LTR and any alteration of this endogenous pattern of chromatin structure that the LTR might introduce. Our analysis has been facilitated by the isolation of the biologically cloned bursal lymphoma cell lines described above (Fig. 2b) which contain only the LTR-associated *c-myc* allele.

The distribution of DNase I hypersensitive sites in these cell lines compared with that in normal bursa is shown in Fig. 3. Normal bursal DNA taken from DNase I-treated nuclei (as used

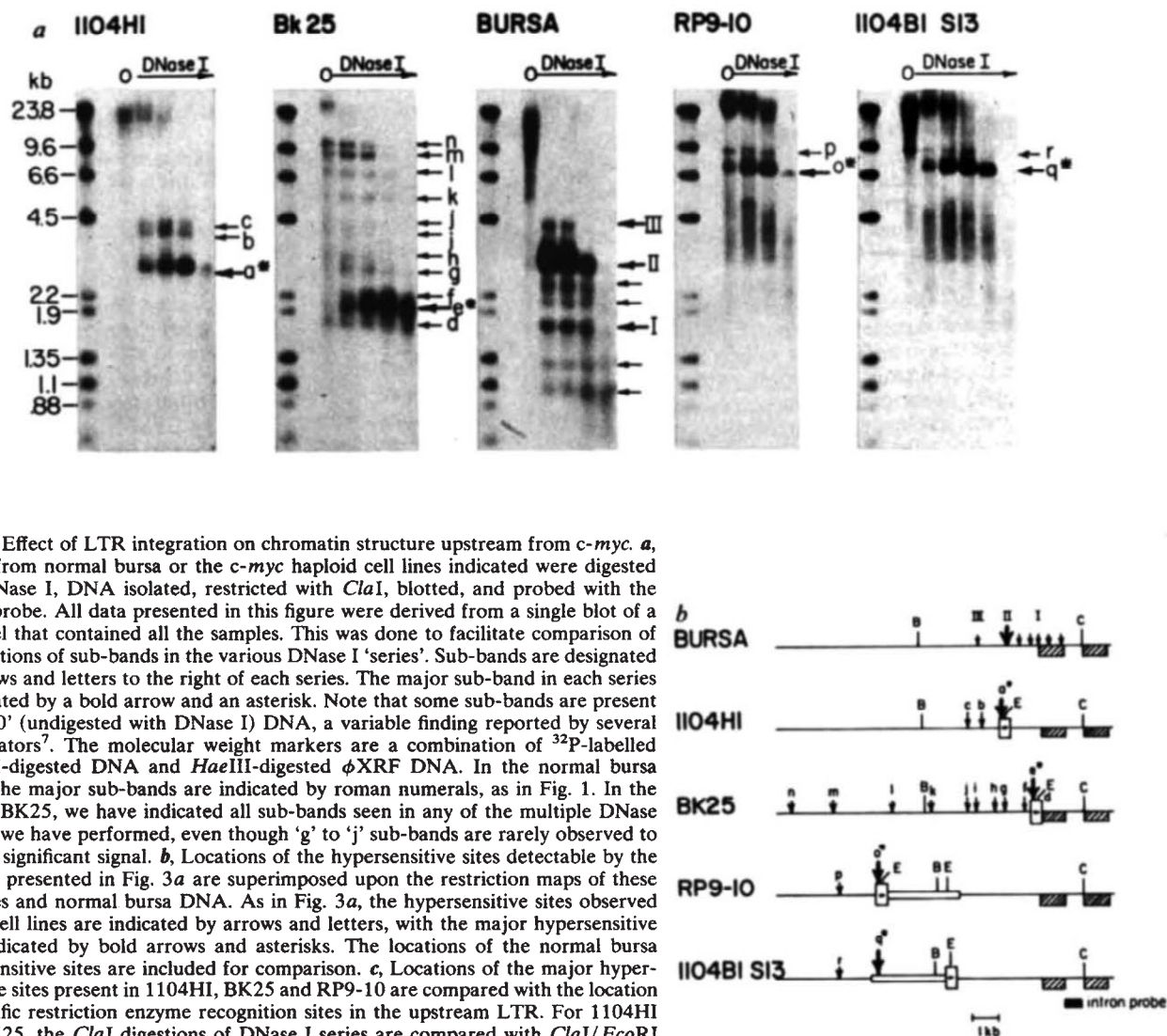
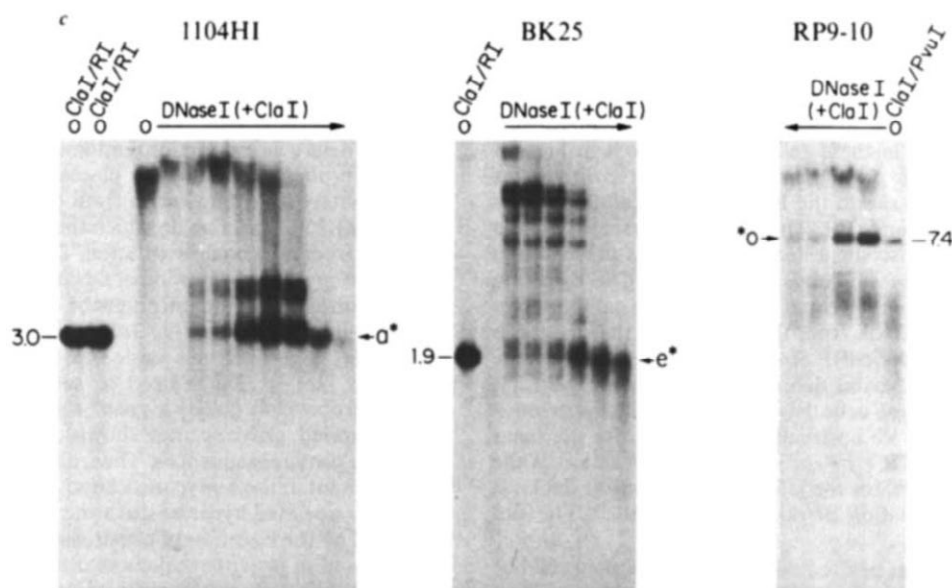


Fig. 3 Effect of LTR integration on chromatin structure upstream from *c-myc*. **a**, Nuclei from normal bursa or the *c-myc* haploid cell lines indicated were digested with DNase I, DNA isolated, restricted with *Cla*I, blotted, and probed with the intron probe. All data presented in this figure were derived from a single blot of a large gel that contained all the samples. This was done to facilitate comparison of the locations of sub-bands in the various DNase I 'series'. Sub-bands are designated by arrows and letters to the right of each series. The major sub-band in each series is indicated by a bold arrow and an asterisk. Note that some sub-bands are present in the '0' (undigested with DNase I) DNA, a variable finding reported by several investigators⁷. The molecular weight markers are a combination of ³²P-labelled *Hind*III-digested DNA and *Hae*III-digested ϕ XRF DNA. In the normal bursa series, the major sub-bands are indicated by roman numerals, as in Fig. 1. In the case of BK25, we have indicated all sub-bands seen in any of the multiple DNase I series we have performed, even though 'g' to 'j' sub-bands are rarely observed to contain significant signal. **b**, Locations of the hypersensitive sites detectable by the analysis presented in Fig. 3a are superimposed upon the restriction maps of these cell lines and normal bursa DNA. As in Fig. 3a, the hypersensitive sites observed in the cell lines are indicated by arrows and letters, with the major hypersensitive sites indicated by bold arrows and asterisks. The locations of the normal bursa hypersensitive sites are included for comparison. **c**, Locations of the major hypersensitive sites present in 1104HI, BK25 and RP9-10 are compared with the location of specific restriction enzyme recognition sites in the upstream LTR. For 1104HI and BK25, the *Cla*I digestions of DNase I series are compared with *Cla*I/*Eco*RI doublet digests of DNA, whereas for RP9-10, a *Cla*I-digested DNase I series is compared with a *Pvu*I/*Cla*I double digest of DNA. See text for rationale and details of this analysis. All blots are hybridized by the intron probe.



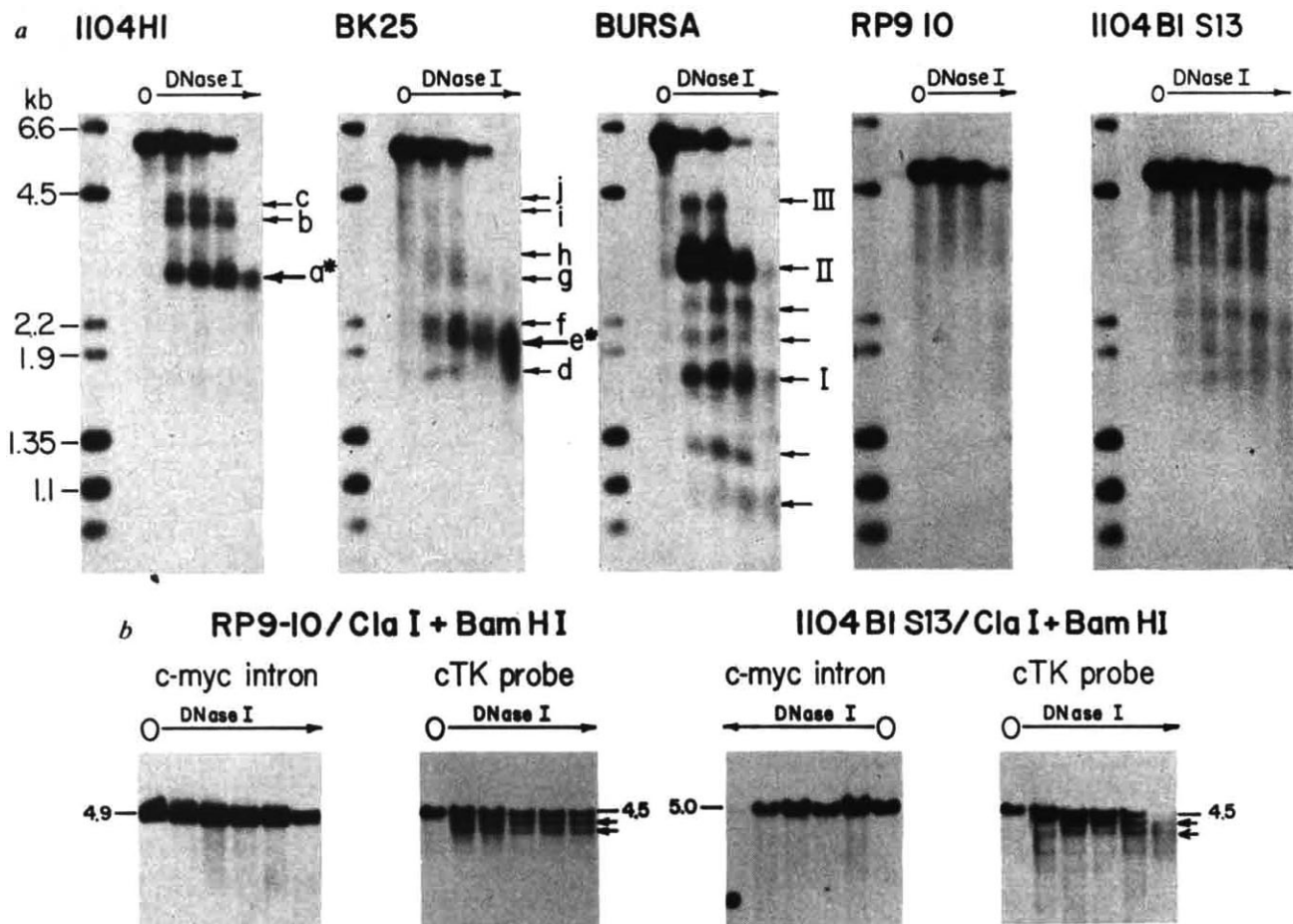


Fig. 4 Suppression of normal *c-myc* hypersensitive sites is associated with the upstream insertion of proviral sequences in different transcriptional orientations. **a**, DNA from the DNase I 'series' described in Fig. 3a was digested with *Cla*I plus *Bam*HI, blotted and hybridized to the intron probe. As described in Fig. 3a legend, all of these samples were loaded on the same gel and transferred to a single blot, to facilitate comparison. Arrows, letters, roman numerals and molecular weight markers are as for Fig. 3a. **b**, DNA derived from DNase I-digested nuclei of RP9-10 (a different preparation from that illustrated in Figs 3a and 4a) and 1104BI S13 were restricted with *Cla*I and *Bam*HI, blotted and hybridized to either the *c-myc* intron probe or a probe for the chicken thymidine kinase (*tk*) gene (a generous gift of M. Wigler). While no hypersensitive sites are apparent in these digests with the intron probe, two prominent hypersensitive sites (arrows to the right of the blots) are seen with the *tk* probe.

in Fig. 1) was digested with *Cla*I, Southern blotted, and probed with the intron probe (Fig. 3a). The results of such digests confirm the observations described above concerning the presence and location of hypersensitive sites upstream from *c-myc*. DNAs from DNase I-treated nuclei isolated from 1104HI and BK25 were digested with *Cla*I and probed with the intron probe. Applying the same reasoning used in the analysis of normal bursa in Fig. 2, this will define DNase I-hypersensitive sites upstream from *c-myc* in these cell lines. As shown in Figs 3a and schematically in 3b, prominent DNase I hypersensitive sites occur near the *Eco*RI site in the LTR of these cell lines (sites *a** and *e** in Fig. 3a and 3b). That these hypersensitive sites reside in the LTR is also suggested by the results presented in Fig. 3c. In this figure the sub-bands, generated by *Cla*I digestion of the DNA from DNase I-treated nuclei of these cell lines, are compared to control DNA from these cell lines that has been digested with *Cla*I and *Eco*RI. Since *Eco*RI cleaves within the LTR, the *Cla*I/*Eco*RI band defines the location of the LTR relative to *c-myc* in these cells. By this analysis, the hypersensitive site lies about 0.1 kb upstream from the *Eco*RI site, near position 100 of the LTR (± 60 bp)^{31,40}. Rehybridization of the blot with probes specific for the U5 and U3 regions of the LTR has confirmed the location of this site within the LTR (not shown).

A similar analysis was performed for another haploid cell line RP9-10. These cells are unusual in that they contain a large

amount of proviral DNA upstream from *c-myc*, including *gag* sequences that contain an *Eco*RI site. Thus, in this analysis, the *Pvu*I site unique to LTR sequences⁴⁰ was used to define the location of the LTR. Upon *Cla*I digestion of a DNase series from RP9-10 cells, a sub-band of 7.5 kb (site *o** in Fig. 3a, b) is seen that corresponds rather closely to the location of the LTR in these cells, as defined by *Pvu*I/*Cla*I double digest of control RP9-10 DNA. This analysis and extensive mapping, using a variety of restriction endonucleases and cellular and proviral probes (not shown), places the hypersensitive site approximately 0.1 kb upstream from position 39 of the LTR.

In 1104BI S13 cell line, in which the LTR has inserted in the opposite transcriptional orientation, 2.7 kb upstream from the 5' end of *c-myc*, *Cla*I digestion of DNase I-treated nuclear DNA and hybridization to the intron probe demonstrates a sub-band of approximately 7.5 kb (Fig. 3a, sub-band *q**, Fig. 3b, site *q**). After *Cla*I digestion, this same 7.5-kb sub-band is detected when the blot is rehybridized to several proviral sequence-specific probes, but not by a probe for cellular sequences 5' to the integrated provirus (not shown), indicating that the site resides in proviral sequences. Thus, although no hypersensitive site is present in the *c-myc* associated LTR in these cells, a new proviral-associated hypersensitive site has been introduced into this locus by the insertion of proviral sequences. Hypersensitive sites located in proviral sequences other than LTRs have been observed previously in avian retroviruses¹¹. Interestingly, as can

be seen in Fig. 3a and b, the provirus-associated hypersensitive sites in RP9-10 and 1104BI S13 (o^* and q^* , respectively) are in similar locations relative to *c-myc*, even though the proviruses are integrated in opposite orientations. Similarly, sites 'p' in RP9-10 and 'r' in 1104BI S13 which are absent in normal bursa (Fig. 3b), are located in comparable regions upstream from the provirus in these cell lines.

Newly integrated proviral sequences appear to affect the local structure of chromatin in surrounding cellular sequences. For example, none of the hypersensitive sites characteristic of the *c-myc* region in normal cells is observed in the RP9-10 cell line upon digestion of DNase I-treated nuclear DNA with *Clal* (Fig. 3a). Since the LTR hypersensitive site is apparent in the RP9-10 chromatin, the lack of the normal *c-myc* sites is not a consequence of our failure to digest these nuclei in a fashion sufficient to detect these sites. Digestion of these same DNA samples with *Clal* and *Bam*HI (*Bam*HI cleaves within the *gag* region of the provirus, downstream of the LTR; see Fig. 2b), and hybridization to the *c-myc* intron probe, confirms the absence of hypersensitive sites in the *c-myc* region between the newly integrated proviral DNA sequences and the first *c-myc* exon (Fig. 4a). In contrast, multiple hypersensitive sites are seen in bursa, 1104HI and BK25 when DNA from DNase I-digested nuclei from these cells is digested with *Clal* and *Bam*HI and blot hybridized to the intron probe (Fig. 4a). The absence of these sites in RP9-10 is also seen in a *Clal*/*Bam*HI digestion of an expanded DNase I series from another preparation of these cells (Fig. 4b). As a control, multiple hypersensitive sites around the chicken thymidine kinase (*tk*) gene are detected when this blot is rehybridized to a TK probe (Fig. 4b). A similar loss of the normal *c-myc* hypersensitive sites is observed in the 1104BI S13 cell line when DNA from the DNase I-treated nuclei of these cells is digested with *Clal* (Fig. 3a) or *Clal* and *Bam*HI (Fig. 4a and b) and the subsequent blots are hybridized to the *c-myc* intron probe. As described above, in 1104BI S13 the proviral sequences are inserted in the 'opposite' orientation; thus, the *Bam*HI site is in the *gag* gene, downstream from the newly introduced hypersensitive site in this cell line, and defines the 5' end of the *Bam*HI/*Clal* parent fragment (see Fig. 2b). As in the case of the RP9-10 samples, when the 1104BI S13 blot is rehybridized to the *tk* probe, multiple hypersensitive sites are detected (Fig. 4b). Thus, in both of these cell lines, the integration of proviral sequences appears to alter the endogenous structure of the *c-myc* locus, as shown by the loss of DNase I-hypersensitive sites downstream from the newly integrated proviral sequences, and the introduction of new hypersensitive sites into this chromosomal domain.

The analysis of the effect of proviral insertion on the endogenous *c-myc* chromatin structure in the BK25 and 1104HI cell lines is more complex than that for RP9-10 and 1104BI S13, due to the position of insertion of the LTRs in two former cell lines. As described above, both of these cell lines contain hypersensitive sites in the LTR upstream from the first *c-myc* exon (sites a^* and e^*). In 1104HI, the LTR hypersensitive site is approximately 1.2 kb upstream from the 5' end of *c-myc* and in BK25, the LTR site is approximately 100 bases upstream from *c-myc*. Since in both cell lines, approximately 350 bp of proviral information has been inserted, the major endogenous site found in normal cells, which usually resides at 1.2 kbp upstream from the 5' end of *c-myc*, would be displaced to approximately a position 1.55 kbp upstream of *c-myc*, and appear as a sub-band in a *Clal* or *Clal*/*Bam*HI digest of BK25 and 1104HI of 3.3–3.4 kbp (1.55 kbp + 1.8 kbp from the *Clal* site to the 5' end of the first *c-myc* exon). As is evident in Fig. 3b and c, no such sub-band is observed in either cell line. In 1104HI, two additional hypersensitive sites located upstream from the LTR are revealed. The sizes of these sub-bands localize them to 2.3 and 1.8 kbp 5' to *c-myc*. While it is possible that the latter sub-band could correspond to the normal, major site (if our measurements are in error by ± 300 bp), we feel this is unlikely. Clearly, however, the complexity of sites seen in the normal *c-myc* chromosomal domain are absent in the 1104HI

cells. In BK25, multiple hypersensitive sites are observed upstream from the LTR insertion (Fig. 3a, b, c). As in 1104HI, however, none of these map to the locations of the major sites observed prior to the LTR insertion. Thus, in both of these cell lines, insertion of a proviral LTR is associated with the introduction of a new hypersensitive site within the proviral sequences, as well as the suppression of some endogenous *c-myc* hypersensitive sites and the uncovering of other 'cryptic' sites in the 5' flanking region of the *c-myc* gene.

Discussion

We have described the normal pattern of DNase I hypersensitive sites that lie upstream from *c-myc* in normal chicken cells. Major DNase I hypersensitive sites lie near the beginning of the coding region (site I), and 1.2 (site II) and 2.5 kb (site III) upstream from the coding region in every tissue from the various stages of chicken development we have thus far investigated. Interestingly, several investigators have observed a nonrandom distribution of integration sites of the LTR upstream from *c-myc* (refs 32–36, and W. Hayward, personal communication), most lying within an interval of 1.2 kb upstream from the coding region, a region flanked by the DNase I-hypersensitive sites I and II found in normal bursa. These findings raise the possibility that the chromatin in this local region may assume a conformation (as determined by DNase I sensitivity) preferentially targeted for integration within the *c-myc* domain. Clearly, however, we cannot eliminate the possibility that integration occurs in a random fashion in the *c-myc* region, with those insertions at specific sites (for example, upstream from splice donor sequences³⁰) giving these cells a selective growth advantage.

The identification of bursal lymphoma cell lines that are haploid at the *c-myc* locus has allowed us to examine the effects of integration of an ALV proviral LTR in this region. In all of these lines a DNase I hypersensitive site can be identified within the proviral sequences integrated upstream from *c-myc*. In three cases (RP9-10, BK25, 1104HI) the hypersensitive site is located between positions 25 and 150 of the LTR, a region associated with enhancement of gene expression in injected and transfected *in vitro* constructs^{38,39}. In RP9 cells, a transcript containing proviral and *c-myc* sequences has been described³², indicating that the LTR is acting as a downstream promoter in these cells. However, in 1104HI and BK25, the amplified *c-myc* transcripts lack LTR sequences³²; thus, in these cases, the observed enhancement of *c-myc* expression does not appear to be the result of downstream promotion, and probably reflects an enhancing effect of the LTR. These results suggest a correlation between those sequences which have enhancer function and the location of the DNase I hypersensitive site in the LTR.

In 1104BI S13 cells, the LTR lies 2.7 kb upstream from *c-myc* in the opposite transcriptional orientation and does not contain a prominent DNase I hypersensitive site as seen in the other cell lines. Despite this, *c-myc* expression is enhanced to high levels and the transcript is identical in size to the normal 2.5-kb transcript identified in fibroblasts¹⁹ and does not contain proviral sequences (M. Linial, personal communication). Thus, in these cells either the LTR or some other proviral sequence is acting as an enhancer over a remote distance^{38,41–43}. Are enhancer sequences and hypersensitive sites, in fact, related? One interpretation of the 1104BI S13 data would be that the enhancer resides in the LTR upstream from *c-myc*, but it lacks a hypersensitive site, possibly due to minor sequence changes. Alternatively, the enhancer in these cells may reside in the proviral sequences defined by the hypersensitive site upstream from the LTR. The latter interpretation would suggest that proviral sequences outside the LTR may contain an enhancer that can be functionally recruited when the enhancer in the LTR is defective, or which is 'dominant' to the LTR enhancer sequences. These possibilities can be tested in various ways using cloned fragments encompassing *c-myc* and proviral sequences from these cell lines.

The data in Figs 3 and 4 suggest that the introduction of proviral sequences upstream from *c-myc* alters the pattern of

endogenous DNase I hypersensitive sites normally found in the upstream region of *c-myc* in uninfected cells. The prominent hypersensitive site 1.2 kb upstream from the coding region (Site II), as well as the other 'minor' sites, are much reduced or absent in RP9-10 and 1104BI S13 cells. In BK25 and 1104HI, the LTR is inserted between the major endogenous *c-myc* hypersensitive site (1.2 kbp upstream from the 5' exon) and the 5' exon; this insertion is associated with the loss of this major endogenous hypersensitive site, as well as the acquisition of new sites within the LTR, and the uncovering of some additional sites in cellular sequences upstream to the insertion sites. The above results raise the possibility that the local alterations of chromatin structure associated with the insertion of the LTR or other proviral sequences may affect the transcriptional activity of *c-myc*. For example, one possibility is that specific sequences within the LTR act as dominant 'sinks' of superhelical torsional stress in the DNA of the *c-myc* locus (see refs 44-46 for discussion of this concept), assayable as DNase I hypersensi-

tive sites. In this view, the loss of the major endogenous *c-myc* hypersensitive site would be the consequence of the reversion of these cellular sequences to the 'normal' B form. Within the context of transcriptional enhancement, the LTR-associated hypersensitive site may represent a conformation of DNA which provides a more efficient entry site than the endogenous *c-myc* hypersensitive site for various transcriptional factors, or may provide a novel nucleation site for the positioning of nucleosomes in this region. This latter possibility could also result in the alteration of the pattern of hypersensitive sites in the *c-myc* locus.

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Induction of altered chromatin structures by simian virus 40 enhancer and promoter elements

Jan Jongstra, Tim L. Reudelhuber, Pierre Oudet, Christophe Benoist, Chi-Bom Chae, Jean-Marc Jeltsch, Diane J. Mathis & Pierre Chambon

Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg Cedex, France

Two simian virus 40 transcriptional promoter elements, the 72-base pair (bp) repeat and the 21-bp repeat region, induce chromatin structures of increased DNase I sensitivity when transposed elsewhere in the viral genome. The induction of a sufficiently long stretch of DNase I-sensitive chromatin leads to the appearance of a visible nucleosome-free region.

LATE in infection, simian virus 40 (SV40) DNA is found in the nucleus of infected cells in the form of a minichromosome (see ref. 1 for references). When nuclei of SV40-infected cells or isolated SV40 minichromosomes are treated with various endonucleases (refs 2-4 and references therein) a region extending for ~350 base pairs (bp) from the unique *Bgl*II site towards the late side is preferentially cut (the ori region; Fig. 1, ORI A). Detailed studies of the products of DNase I digestion early

and late in infection have revealed the existence of several discrete hypersensitive sites within this DNA fragment^{5,6} and electron microscopy has shown that ~25% of the minichromosomes extracted from nuclei late in infection contain a nucleosome-free region (the nucleosome gap) of ~350 bp located in the same position^{3,7}. Studies with viral mutants in which parts of the ori region are duplicated indicate that this region contains the information required for the generation of the nuclease-

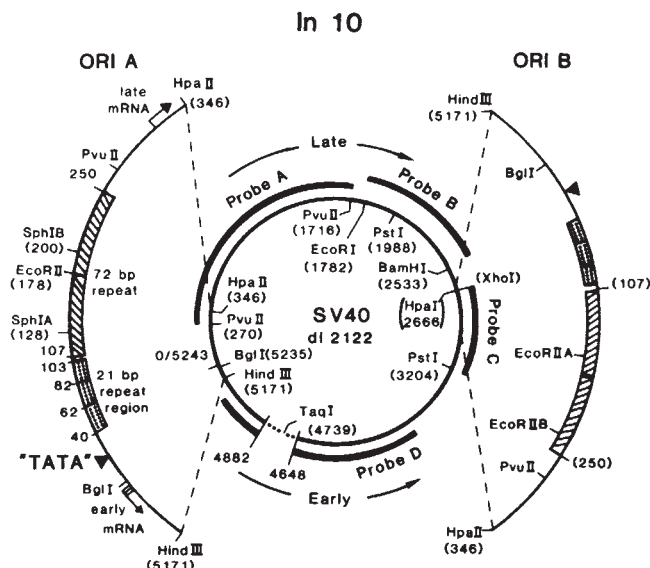


Fig. 1 Schematic representation and construction of the SV40 mutant In10. The inner circle represents the genome of SV40 d12122 which has a 234-bp deletion around the *TaqI* site (dotted line). The position and coordinates of some relevant restriction sites and landmarks are indicated and numbered according to the BBB system¹. The SV40 ori region (ORI A) which contains the origin of replication (around the *BglI* site) and the early and late gene promoter sequences are shown enlarged at the left-hand side. The three early gene promoter elements (TATA box, 21-bp repeat region and 72-bp repeat) are indicated. A similar ori region from *HindIII* to *HpaII* (5,171–346) was inserted at position 2,666 in the same orientation with respect to early gene transcription, as shown enlarged on the right-hand side (ori B). The ori fragment was taken from plasmid pSV1 and contained an 8-bp deletion from position 173 to 181, deleting the *EcoRII* site at position 178 (ref. 12). The solid bars indicate the restriction fragments used as hybridization probes to map regions of increased DNase I sensitivity at ori A and ori B. To generate the SV40 insertion mutant In10, the following plasmids were first constructed: pSV22, a recombinant between the DNA of SV40 d12122 and a *PstI* site-deleted pBR322 joined at their unique *EcoRI* sites; pSV10, the small SV40 *PstI* fragment (1,988–3,204) was inserted in the *PstI* site of pBR322; pSV10IN10, constructed by blunt-end ligation (after DNA polymerase I treatment) of the *HindIII*–*HpaII* ORI fragment (5,171–346) in the unique *HpaI* site present in the SV40 *PstI* fragment of pSV10. The small *PstI* fragment of pSV10IN10 was purified on a sucrose gradient and ligated in pSV22 in place of the original SV40 *PstI* fragment to give pIN10. Virus stocks of SV40 d12122 and In10 were prepared from pSV22 and pIN10 as follows: DNA from plasmids pSV22 and pIN10 was digested with *EcoRI* to separate the SV40 moiety from the pBR322 sequences, ligated at low DNA concentrations (5–10 $\mu\text{g ml}^{-1}$) to circularize the DNA, and transfected into CV-1 cells in the presence of 0.5 mg ml^{-1} DEAE-dextran¹² using 50 ng of ligated DNA per 6 cm Petri dish. Virus was collected by repeated freezing and thawing when 90% of the cells had lysed. Virus stocks were then prepared by infections at low multiplicity with plaque-purified virus and used for subsequent experiments. Recombinant constructions were verified in plasmids by partial DNA sequencing and in virus stocks by detailed restriction analysis of Hirt extracts.

sensitive chromatin structure^{8,9} and the nucleosome gap^{8,10}.

These findings are of particular interest because the ori region contains the DNA sequences involved in initiation of transcription of early and late SV40 genes (see ref. 1) in addition to the viral replication origin¹¹. Recent studies have defined three DNA regions which have a role in promotion of early gene transcription *in vivo*. These are (Fig. 1) the 'TATA box', which dictates the proper initiation site for transcription^{12–14}, the 21-bp repeat region containing six G+C-rich repeated sequence motifs, the deletion of which decreases early gene expression^{12,15,16}, and the enhancer element, the 72-bp repeat. Deletion of one of the two 72-bp sequences has very little effect on early gene expression but deletion of all, or part, of the remain-

ing repeated sequences reduces early transcription considerably^{12,14,17–21}.

Therefore, the early promoter sequences are localized within the same fragment of the SV40 genome that displays an altered chromatin structure. Similarly, a variety of cellular genes show alterations in chromatin structure upstream from the mRNA cap site, which correlate with transcriptional activity (refs 22–25 and references therein). Thus, alterations in chromatin structure, as detected by increased sensitivity to endonucleases and the presence of a nucleosome gap, might be induced by transcriptional promoter elements.

Here we describe experiments which were designed to identify the sequences in the ori region necessary to induce chromatin structures of increased DNase I sensitivity and a nucleosome gap. Our approach was based on the observation that SV40 mutants having deletions in the large-T intron can accommodate extra DNA sequences in the *HpaI* site at position 2,666 (Fig. 1) and still grow normally²⁶. Different parts of the ori region were inserted at this *HpaI* site using the mutant d12122, which has a deletion between positions 4,648 and 4,882 (ref. 27) to create mutants carrying two ori regions: the wild type ori A, and a second wild type or mutated origin region, ori B (Fig. 1).

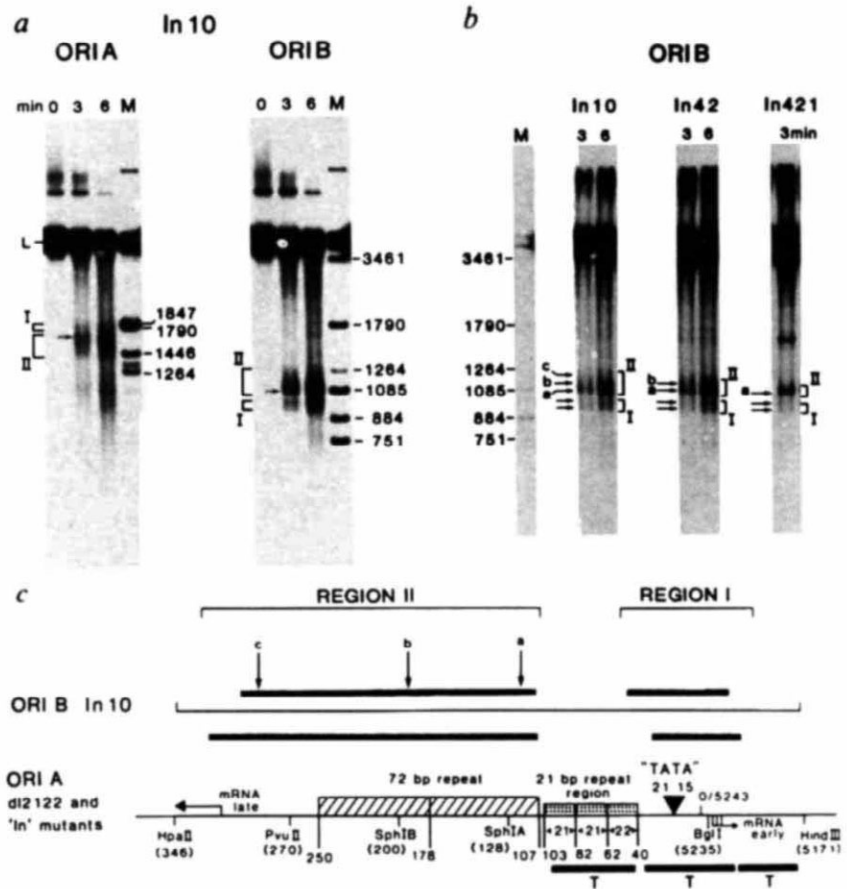
Induction of DNase I sensitivity

Figure 1 describes the double-origin mutant In10 which has the 418-bp *HindIII*–*HpaII* ori fragment (5,171–346; Fig. 1) inserted as a second ori (ori B). To measure the increased DNase I sensitivity at ori A and B, CV-1 cells were infected with an In10 virus stock, nuclei were prepared 40–42 h later, and regions of increased DNase I sensitivity were mapped using an indirect end-labelling technique²⁸. The pattern of DNase I digestion at ori A and B was revealed after hybridization of the blotted DNA to probes A and B, respectively (see Fig. 1 and Fig. 2 legend).

An example of a resulting autoradiogram using probe A is shown in Fig. 2a (ORI A). The heavy band (L) corresponds to In10 DNA which was not digested with DNase I, but only linearized with *EcoRI*. In10 chromatin DNA digested randomly with DNase I gave rise to a continuous smear, the intensity of which increased with increasing time of DNase I digestion. Regions of increased DNase I sensitivity at ori A produced two sets of DNA fragments, labelled I and II. DNA purified from In10-infected cells (or from cells infected with other In mutants described in this study) and digested with DNase I and *EcoRI* showed only the background smear (not shown). The early border of the DNase I-sensitive region II mapped at position 109 ± 25 and thus coincided well with the early border of the 72-bp repeat (Fig. 2c). The late border of region II mapped at position 323 ± 20 . Occasionally, rather than a continuous smear, region II in ori A was resolved into two or three separate sets of fragments of unequal intensity, suggesting the existence of several subregions. The most prominent subregion (arrow in Fig. 2a) was always proximal to the early side of region II. Figure 2a also shows that using probe A, the DNase I-sensitive region I was ~ 30 bp long and centred over position 5,220. However, the presence of the highly DNase I-sensitive region II between region I and the *EcoRI* site used as a reference point for the mapping experiments, might have led to an underestimation of the length and intensity of region I. When the *MboI* site at position 4,099 was used as a reference point using a *MboI*–*AvaII* fragment (4,099–5,118) as probe (probe D, Fig. 1) region I was shown to include the TATA box and extended from position 5,210 to 35. The pattern of DNase I sensitivity determined over ori A in In10 was similar to that found in wild-type SV40, d12122 and all double-origin mutants tested (results summarized in Fig. 2c, 'ORI A'). Similar results were reported by Saragosti *et al.*³ for wild-type SV40.

Figure 2a (ORI B) shows that when probe B was used instead of probe A, a pattern of DNase I sensitivity similar to that seen at ori A was revealed at ori B. Region II at ori B mapped from position 110 ± 25 to 310 ± 20 , while region I mapped from position $5,220 \pm 20$ to 45 ± 20 (Fig. 2c). These locations were

Fig. 2 Mapping of the regions of increased DNase I sensitivity at ori A and ori B of In10, In42 and In421. **a**, Mapping of DNase I-sensitive regions at ori A and ori B was done as follows. CV-1 cells were infected with the appropriate virus stock and collected 40–42 h after infection by scraping them into a small volume of ice-cold buffer A (75 mM KCl, 5 mM PIPES pH 7.0, 150 mM sucrose) containing 1 mM EDTA, then washed twice with the same buffer by centrifugation for 5 min at 3,000 r.p.m. in a Sorvall HB-4 rotor at 4 °C. The cells were lysed in buffer A containing 0.5% Nonidet P-40 for 5 min on ice and the nuclei were washed once with buffer A by centrifugation as described above. The nuclear pellet was resuspended in buffer A at a DNA concentration of 0.5–1.0 mg ml⁻¹. Nuclease digestions were carried out at room temperature by adding 0.1 vol. 10 mM CaCl₂ and DNase I (Worthington; 2,624 U mg⁻¹) to a final concentration of 120 U ml⁻¹ in a total volume of 550 µl. Samples (90 µl) were removed at various times and immediately mixed with 110 µl of 20 mM Tris-HCl pH 7.4, 10 mM EDTA, 0.2% SDS, containing 20 µg proteinase-K and incubated at 37 °C for 2–3 h. The samples were extracted once with 1 vol. phenol-chloroform (1:1) and once with 3 vol. ether. The aqueous phase was digested with RNase A at 10 µg ml⁻¹ for 1 h at 37 °C and extracted with phenol-chloroform and ether, then the DNA was digested with a twofold excess of *EcoRI* (BRL) and layered onto a 2% agarose gel for electrophoresis. DNA was transferred from the agarose gel to a nitrocellulose filter and hybridized according to Wahl *et al.*⁴², but with omission of the dextran sulphate. To map the regions of increased DNase I sensitivity at ori B, the filter was hybridized to probe B (Fig. 1) labelled with ³²P by nick-translation. After dehybridization at 65 °C for 2 h as described by Thomas⁴³, regions of increased DNase I sensitivity at ori A were mapped using probe A (Fig. 1). The time of digestion with DNase I is indicated (in min) at the top of each lane. Lane M contains marker fragments prepared by digesting SV40 DNA with the restriction enzymes: *EcoRI*+*BglI*, *EcoRI*+*BamHI*, *PvuII*, *HinfI* and *MboI*. Lane M contains 50 ng of each digest and the numbers to the right of each panel represent the length (in bp) of the restriction fragments detected by the hybridizing probe. The 884-bp marker fragment visualized by probe B (in ORI B) is the *EcoRI*-*HpaI* fragment (1,782–2,666), which marks the extremity of the inserted fragment proximal to probe B. The DNase I-sensitive regions I and II described in the text are indicated by brackets and a prominent band visible in region II at ori A and ori B is indicated by an arrow. L, linearized viral DNA. In the particular experiment shown here dehybridization was incomplete; this resulted in the appearance of a signal corresponding to ORI B in the panel labelled ORI A below the 1,264-bp marker fragment. **b**, Fine structure in region II at ori B. Samples of In10, In42 and In421 were standardized as described in the text to allow a valid comparison of the DNase I digests. The 3-min digestion points of the three mutants were digested to the same degree at ORI A. Similarly, the 6-min time points for In10 and In42 were directly comparable. The extent of the DNase I-sensitive regions is indicated by brackets labelled I and II and arrows indicate the fine structure within these regions. Lane M contains marker fragments as for **a**. The construction of pIn42 and pIn421 is described in Fig. 5 legend. **c**, Diagrammatic comparison of regions of increased DNase I sensitivity at ori B in In10 and ori A in wild-type and mutant SV40. The lower line (ori A) represents the SV40 ori region with the TATA box, the 21-bp repeat region and the 72-bp repeat. The three solid bars below the line indicate the three T-antigen-binding sites^{32,33}. The solid bars above the lines ori A and ori B in In10 indicate the extent of regions of increased DNase I sensitivity I and II, and are a summary of several mapping experiments using probes A and D to analyse ori A, and probes B and C to analyse ori B. The vertical arrows indicate the approximate location of the bands a, b and c shown in **b**.



confirmed using probe C and the *PstI* site (3,204; Fig. 1) as a reference point (not shown). Several similarities in the digestion patterns at ori A and ori B in In10 are noteworthy. First, both regions of DNase I sensitivity at ori B mapped over the same DNA sequences as at ori A. Second, region II at ori B was more sensitive to digestion with DNase I than region I; this confirmed that the increased DNase I sensitivity of region II compared with region I at ori A (see above), was not due to the use of a probe from the late side which might have led to a preferential mapping of DNase I cut sites proximal to the *EcoRI* site. Third, region II at ori B also showed a fine structure with a prominent band mapping close to the early side of the 72-bp repeat (arrow in Fig. 2a, ORI B). Figure 2b gives the results of an experiment showing more clearly the presence of five bands at ori B in In10 (arrows), two of which mapped within region I and three within region II.

To investigate which sequences were responsible for the induction of region II and the prominent cuts contained within it, we constructed mutants In42 and In421, which contain

HindIII-*PvuII* ori fragments (5,171–270) with an intact 72-bp repeat or only one 72-bp sequence, respectively (Fig. 5a). To validly compare the DNase I digestion patterns at ori B for the different mutants, we first ensured that all samples were equilibrated for viral yield and degree of digestion. To this end, different amounts of total DNA extracted from infected cells were loaded on the gel and then only those samples which showed identical signals for the linear SV40 DNA (band L in Fig. 2a) and an equal degree of digestion at ori A as determined by hybridization with probe A, were compared at ori B using probe B (the same standardization procedure was used for all the experiments described below). The absence of the *PvuII*-*HpaII* fragment (270–346) at ori B in In42 resulted in the appearance of a shorter region II lacking band c (Fig. 2b). Additional deletion of one 72-bp sequence (In421) shortened region II even further and eliminated band b (Figs 2b, 5a). From these results, together with the data of several mapping experiments, we conclude that bands a and b fall on the early side of each 72-bp sequence and band c on the late side of the

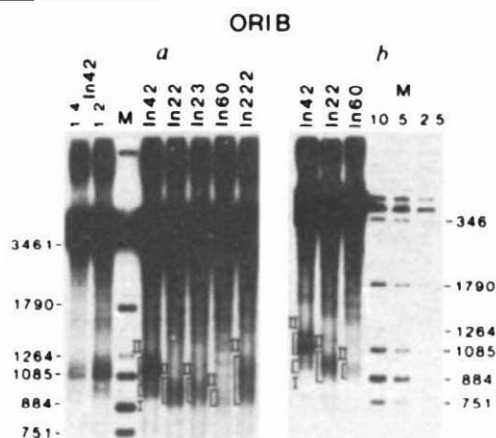


Fig. 3 The 72-bp repeat induces an altered chromatin structure over itself. *a*, The DNase I digestion patterns at ori B of different In mutants as indicated at the top of each lane, were visualized using probe B (see Fig. 1) and the extent of regions of increased DNase I sensitivity are indicated by brackets. Samples were standardized as described in the text. To estimate the magnitude of the differences in intensity of region II in In42, In22 and In23, twofold and fourfold dilutions of the In42 sample shown in the central panel were included (In42 diluted 1:2 and 1:4). Lane M contains markers as described for Fig. 2a. The samples were digested at ori A to ~5% (as determined by the ratio of the signals corresponding to linear SV40 and to DNase I cuts at ori A). *b*, A different set of standardized samples digested at ori A to ~25% was run on a separate gel in the presence of different amounts of marker fragments (lanes M containing 10, 5 or 2.5 μ l of the same marker mix). The construction of the In mutants is described in Fig. 5 legend.

*Pvu*II (270) restriction site. In all three mutants In10, In42 and In421, region II was of equal intensity and was always more sensitive than region I.

The relationship between bands a, b and c and the specific cuts made at the wild-type SV40 ori region at positions 370, 270 and 190 as reported by Cremisi⁵ is unclear, but the differences in position may be related to the use of rather long fragments starting from the *Eco*RI site to probe the structure of the ori region. The use of shorter probes localized nearer to the ori region of wild-type SV40 has allowed the identification of as many as five specific cuts within region II⁶, three of which were mapped at positions 110, 185 and 290 and thus may correspond to the bands a, b and c described here. The two additional cuts which were mapped by Cereghini *et al.*⁶ within the 'late' moiety of each 72-bp sequence probably reflect the higher degree of resolution obtained in their assay. As specific bands within regions of increased DNase I sensitivity result from the cuts made at hypersensitive sites at an early stage of DNase I digestion, it is possible that the exact number and location of such cuts differ with experimental conditions and may depend on various parameters such as temperature, digestion buffer, amount of DNase I or method of preparation of nuclei.

Induction of DNase I sensitivity by enhancer

To further define the sequences necessary to induce the increased DNase I sensitivity over region II, we inserted in both orientations at ori B a DNA fragment from position 113 to 270, containing mainly the 72-bp repeat (In22 and In23; Fig. 5a). Both mutants displayed a DNase I-sensitive region of similar intensity over the inserted fragment (see Fig. 3a, region II). This sensitive region differed from the region II in In42 in several ways. First, regions II in In22 and In23 seemed to be less intense than region II in In42. To estimate the magnitude of this difference, several dilutions of the In42 sample were included on the same gel. From a comparison of these samples, we estimated the intensity of regions II in In22 and In23 to be 2–3-fold less than in In42 (Fig. 3a, In42 diluted 1:2 and 1:4). Second, regions II in In22 and In23 appeared more diffuse, and third, although some fine structure was visible in region II in In22 and In23, no prominent band close to the early border of

the inserted 72-bp repeat was observed. The decreased DNase I sensitivity of the isolated 72-bp repeat at ori B in In22 and In23 (compared with region II at ori B in In42) could be due to the absence of certain ori sequences on the early side of this repeat or could be related in some way to the smaller size of the insert. To test the effect of the size of the insert, we took advantage of a uniquely reconstructed *Xho*I site at one end of ori B in In22 to insert a second 72-bp repeat (from position 113 to 270), thus constructing In222 carrying an insert of approximately the same length as In42 (Fig. 5a). Figure 3a shows that a duplication of the DNase I-sensitive region seen in In22 was present at ori B of In222, but of the same intensity as in In22 or In23. This suggested that sequences between the *Hind*III site (5,171) and position 113 affected the intensity of the DNase I sensitivity generated over the 72-bp repeat.

We also tested whether a single 72-bp sequence was able to induce an altered chromatin structure by deleting one of the two 72-bp sequences from ori B of In22 (In60, Fig. 5a). The remaining region of increased DNase I sensitivity at ori B was shorter than in In22 or In23 and also seemed less intense (Fig. 3a). To ensure that these differences in intensity were not due to different digestion kinetics, we prepared another set of standardized samples from In42, In22 and In60 (Fig. 3b) which were more heavily digested with DNase I than the set shown in Fig. 3a. Using the marker lanes as standards, we again estimated that region II in In22 was 2–3-fold less intense than in In42 and that the DNase I sensitivity induced at ori B in In60 by a single 72-bp sequence was reduced ~2–3-fold compared with the intact 72-bp repeat (In22) or a dimer of this repeat (In222). This latter decrease might be due to the small size of the insert in In60 rather than be related to the deletion of specific sequences. Also, note that the insert at ori B in In60 consists of slightly less than an intact 72-bp sequence since its early border is at position 113.

Two reports which appeared when this work was in progress have dealt with the effects of the 72-bp repeat on chromatin structure using SV40 mutants containing transposed ori fragments. Gerard *et al.*⁹ concluded that this repeat was not in itself sufficient for the induction of increased nuclease sensitivity. These authors used an ori fragment containing a single 72-bp sequence and might, therefore, have been unable to detect the increase in nuclease sensitivity induced by this fragment (compare In22 and In60, Fig. 3a, b). The results given in Fig. 3a clearly demonstrate that the 72-bp repeat induces an increase in DNase I sensitivity independent of its orientation (In22 and In23). This is in contrast to the findings of Fromm and Berg²⁹ who inserted an ori fragment (from position 95 to 270) into the *Hpa*I site (2,666) of a SV40 genome in which a fragment from position 73 to 270 had been deleted from the wild-type ori region. They found that the 95–270 fragment induced an increase in DNase I sensitivity only when inserted in the orientation comparable to ori B in In23. This discrepancy could be due to the small difference in the sequences of the ori fragments tested in the two studies (base pairs 113 to 270 in our study) or to other differences in the recombinant constructions.

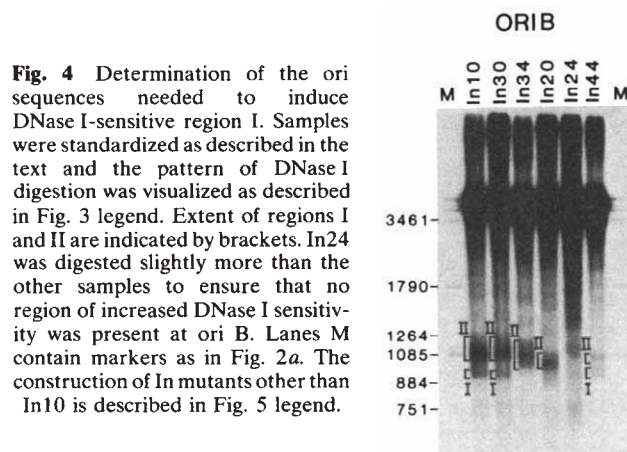


Fig. 4 Determination of the ori sequences needed to induce DNase I-sensitive region I. Samples were standardized as described in the text and the pattern of DNase I digestion was visualized as described in Fig. 3 legend. Extent of regions I and II are indicated by brackets. In24 was digested slightly more than the other samples to ensure that no region of increased DNase I sensitivity was present at ori B. Lanes M contain markers as in Fig. 2a. The construction of In mutants other than In10 is described in Fig. 5 legend.

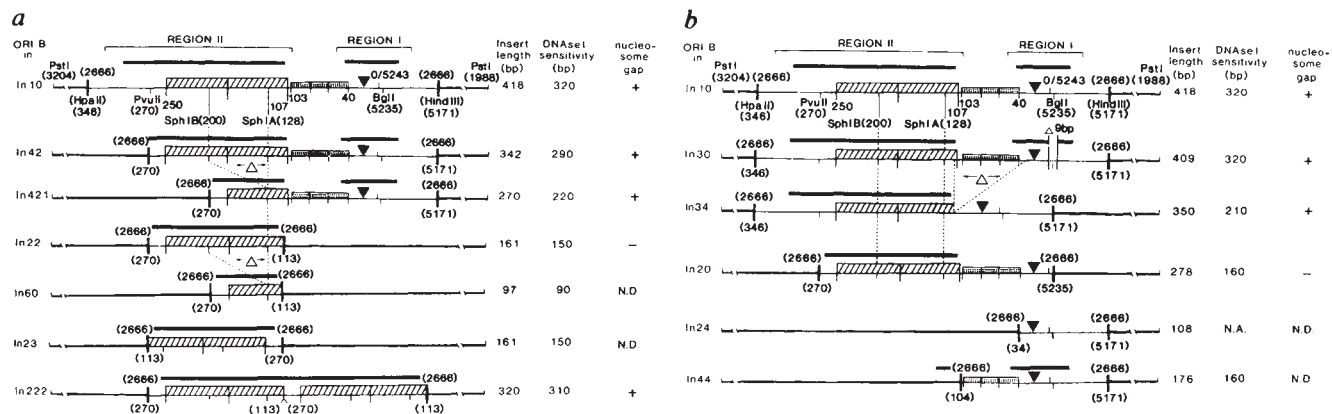


Fig. 5 Diagrammatic representation of the results obtained with the various In mutants. The small *Pst*I fragments containing ori B are shown (see Fig. 1). For each mutant the insert at ori B is represented by a thin horizontal line bounded by thick vertical bars. The extent of the regions of increased DNase I sensitivity (I and II) are indicated by black horizontal bars as in Fig. 2c. The column 'DNase I sensitivity' gives the distance (in bp) between the outer extremities of the domain of DNase I-sensitive chromatin. Approximately 25% of the minichromosomes extracted from cells infected with any of the In mutants exhibited at least one nucleosome gap. More than 150 minichromosomes containing at least one gap of those mutants for which + appears in the right-hand columns of *a* and *b* were examined to determine the percentages of minichromosomes exhibiting two gaps—these were as follows: for In10, 32%; In34, 30%; In30, 27%; In42, 22%; In421, 28% and for In222, 24%. When no minichromosomes containing two gaps were seen (indicated by -) more than 300 minichromosomes containing a single gap were examined. N.A., not applicable; N.D., not determined. *a*, Summary of results defining ori sequences needed to induce region II. In42, In22 and In23 were constructed by directly inserting the appropriate ori fragments in pSV22X, a derivative of pSV22 (see Fig. 1 legend) in which the *Hpa*II site at position 2,666 was converted to a unique *Xho*I restriction site by inserting a *Xho*I linker. In421 was derived from In42 by deletion of one 72-bp sequence between *Sph*I A and *Sph*I B at ori B. The insert in In22 is the pHS102 fragment A (113–270) described by Moreau *et al.*¹⁷; the same insert is present at ori B in In23 in the opposite orientation. In60 is derived from In22 by deletion of the *Sph*I A–*Sph*I B fragment at ori B. In222 was constructed by inserting a second 72-bp repeat (113–270) in the *Xho*I site reconstituted at the 'late' side of ori B in In22. *b*, Summary of results defining ori sequences necessary to induce region I. In20 was constructed in a way similar to In10 (as described in Fig. 1 legend) using a purified *Bgl*II–*Pvu*II fragment (5,235–270) derived from pSV1. The other In mutants were constructed as described above for In42 using the following ori fragments: the *Hind*III–*Hpa*II fragment (5,171–346) from pAS¹² which has a 9-bp deletion around the *Bgl*II site (In30) or from pMD102¹⁵ which has a deletion between positions 34 and 113 (In34), or the *Hind*III–*Bam*HI fragments from pRE7 (5,171–34; In24) or from pMKD231 (5,171–104; In44) described by Everett *et al.*¹⁵.

Importance of 21-bp repeat region

As DNase I-sensitive region I maps over the origin of replication proper¹¹, we investigated whether an intact replication origin at ori B was necessary for the generation of region I. ori B in In30 is similar to ori B in In10 (Fig. 1) but has a 9-bp deletion around the *Bgl*II site (see Fig. 5b), which is known to abolish SV40 replication³⁰. Although this deletion had no effect on the induction of DNase I-sensitive region I or II (Figs 4, 5b), insertion at ori B of the *Bgl*II–*Pvu*II fragment (5,235–270), which lacks the early moiety of the sequences contained within region I (In20; Fig. 5b) but not the TATA box, did not generate a stretch of increased DNase I sensitivity corresponding to region I (Fig. 4). Comparing these results with those obtained using In42 (carrying the *Hind*III–*Pvu*II fragment at ori B; see Figs 2b, 5a) indicated that the generation of region I required the presence of sequences between the *Hind*III and *Bgl*II sites. In addition, the insert at ori B of In20 induced a region II which mapped from position 110 ± 10 to 265 ± 15 (Figs 4, 5b), thus confirming the above conclusion that sequences between *Pvu*II (270) and *Hpa*II (346) are important in defining the late border of region II.

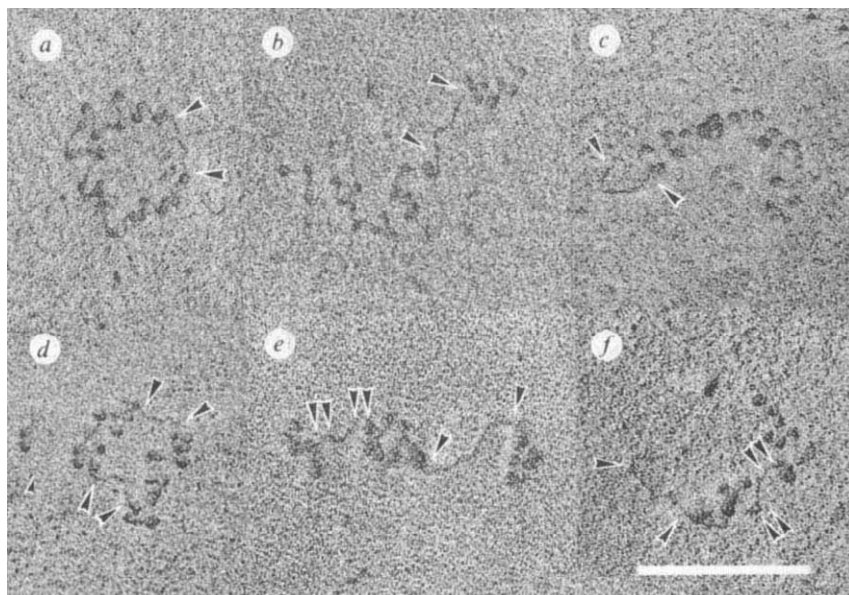
To determine whether, as in the case of region II, the sequences within region I were able to induce DNase I sensitivity over themselves, we constructed In24 which carried at ori B the fragment from position 5,171 to 34 and contained essentially all the DNA sequences included in region I (In24; Fig. 5b). No increase in DNase I sensitivity was induced over this insert (Fig. 4). When we tested In44, which contained at ori B a fragment spanning positions 5,171 to 104, encompassing as in In24 all sequences contained within region I plus the 21-bp repeat region, we observed two regions of increased DNase I sensitivity (In44; Figs 4, 5b). one region mapped from position $5,230 \pm 10$ to 60 ± 10 and was therefore similar to region I at ori A, while a second narrow region mapped just outside the insert on its 'late' side. Both regions of increased DNase I sensitivity at ori B in In44 were of lower but equal intensity (Fig. 4), compared

with the characteristic wild-type pattern at ori A in In44 or at ori B in In10, In30, In42 or In421. These results suggested that DNA sequences localized between positions 34 and 104 (that is, the 21-bp repeat region) were responsible for the induction of region I over the ori sequences located directly on its early side, and might also influence the pattern of increased DNase I sensitivity over the 72-bp repeat which in the wild-type ori is located directly on its late side. Both suggestions were confirmed by the results obtained at ori B in In34, which was similar to In10 but lacked the 21-bp repeat region (Fig. 5b). Not only was region I absent at ori B in In34, but inspection of several autoradiograms similar to that shown in Fig. 4 revealed also that band b of region II in In34 was more intense than band a, in contrast to the relative intensity of these bands in region II of In10 (Fig. 2b).

Induction of a nucleosome gap

About 25% of wild-type SV40 minichromosomes extracted from nuclei late in infection contain a nucleosome gap over their ori region. To investigate whether this gap could be observed at ori B, minichromosomes were extracted from CV-1 cells 40–42 h after infection with an In10 virus stock. Approximately one-quarter of the minichromosomes had a nucleosome gap (Fig. 6a) which appeared to map preferentially over ori A when using the single-cut restriction enzymes *Eco*RI (Fig. 6b) or *Msp*I (Fig. 6c). (Note that the *Hpa*II–*Msp*I site was lost at ori B.) In addition, two diametrically opposed gaps were observed in approximately 7% of the minichromosomes (Fig. 6d). These two gaps were mapped over ori A and ori B after digestion with *Eco*RI (Fig. 6e) or *Msp*I (Fig. 6f). To determine which ori sequences were responsible for the generation of the gap, we analysed most of the 'In' mutants described above for the presence of a gap at ori B. The results, summarized in Fig. 5a,b indicate that a similar fraction of the minichromosomes of the two mutants In 42 and In421 exhibited two gaps, mapping over ori A and ori B, while mutants In20 and In22 exhibited a gap only over ori A. The absence of a visible gap over ori B in In20

Fig. 6 Electron microscopic visualization of In10 minichromosome. Minichromosomes were extracted and purified from CV-1 cells 40–42 h after infection as described elsewhere⁴⁴. Aliquots from the 75S peak fraction were digested with *Eco*RI (b and e) or *Msp*I (an isoschizomer of *Hpa*II; c and f) after addition of $MgCl_2$ to a final concentration of 5 mM. The digestion was stopped by adding 5 mM EDTA. Undigested minichromosomes (a and d) and the digested samples were diluted at least 10-fold in water and 10- μ l aliquots were deposited on carbon-coated grids which had been positively charged by glow discharge in the presence of amylamine. After 1 min of adsorption, the grids were rinsed for 5 min with water then stained for 10 s in 0.1% aqueous uranyl acetate. The grids were then washed for 5 s with water, air-dried and shadowed as described previously⁴⁴. Scale bar in f represents 0.25 μ m. The positions of nucleosome gaps were mapped on minichromosomes cut with a restriction enzyme by counting the nucleosomes between the gap and the extremities of the linearized molecules. a, b, c Show minichromosomes containing one nucleosome gap which mapped over ori A. d, e, f Show minichromosomes containing two gaps. In e and f the gaps indicated by single arrowheads map over ori A and the gaps indicated by double arrowheads map over ori B.



was not due to the smaller size of the inserted fragment, as the insert at ori B in In421 was of a similar size. A possible means of accounting for these results is to postulate that the presence of DNase I-sensitive region I was a prerequisite for the observation of a gap. However, when we examined minichromosomes from In34 which showed no region I at ori B, we found a gap over ori B (Fig. 5b). Alternatively, the induction of a visible nucleosome gap might require the existence of a minimal length of altered chromatin structure which could be made up of either a complete region II as in In34 or a truncated region II plus an adjacent region I as in In42 or In421. To test this hypothesis, we examined minichromosomes extracted from In222-infected cells and found that a fraction of them contained two gaps (Fig. 5a). These results strongly support the conclusion that the total length of increased DNase I sensitivity at ori B is the crucial factor in allowing gap formation, and that the nuclease-sensitive structure and the nucleosome gap occur on the same minichromosomes. In fact, we have recently determined that the visible gap region of isolated wild-type minichromosomes is preferentially excised with *Bgl*II and *Msp*I restriction enzymes (E. Weiss *et al.*, in preparation).

Discussion

Our experiments, reported here, demonstrate that generation of the full pattern of increased DNase I sensitivity at the SV40 ori region depends on the presence of multiple ori sequences and on their precise arrangement. First, the generation of a full-length region II depends on both sequences within the 72-bp repeat and a separate set of sequences located between the *Pvu*II (270) and *Hpa*II (346) sites (compare In10, In42 and In44). Second, region I is induced only when the 21-bp region, which is itself resistant to DNase I digestion, is present together with sequences between the *Hind*III (5,171) and *Bgl*II (5,235) sites (compare In10, In34 and In20). Third, the 21-bp repeat region has a distinct qualitative effect on the generation of increased DNase I sensitivity over the 72-bp repeat, leading to a well defined 'early' border and a prominent subregion (band a; compare In10 and In34). Studies using mutated 21-bp repeat regions are required to investigate whether the effects of this region on chromatin structure are related to its promoter activity for early^{12,14–16} or late¹⁹ genes and whether its resistance to DNase I digestion is due to the binding of a transcription factor³¹ or of T-antigen^{32,33}. In any case, a large fraction of the increased DNase I sensitivity at ori is critically dependent on the presence of the 72-bp repeat (compare In10, In42, In421 and In44). Most important, an alteration of chromatin structure is gener-

ated at ori B by insertion, in either orientation, of the 72-bp repeat region, in the absence of other ori sequences (In22 and In23).

Our results also indicate that the formation of a visible nucleosome gap is correlated with the generation of a sufficiently long stretch of altered chromatin structure, the minimal length of which seems to be between 160 and 210 bp (see Fig. 5a,b). This can be achieved in various ways, but the major conclusion is that sequences mostly contained within the 72-bp repeat are sufficient to generate a visible nucleosome gap (see In222). It is possible that a single 72-bp repeat could generate a nucleosome gap which is not detectable because its length is too close to the maximal size of the variable internucleosomal linker length of spread SV40 minichromosomes^{1,3}.

The SV40 72-bp repeat has been characterized as a *cis*-acting bidirectional enhancer of transcription from potential promoter elements²¹. The observations that proximal potential promoter elements are preferentially activated and that activation of transcription from a given promoter element decreases as the length of interposed sequences increases, have suggested that the SV40 enhancer acts like a bidirectional 'entry site' for some element(s) of the class B transcription machinery^{17,21}. Our present results suggest that the generation of an altered chromatin structure by the SV40 enhancer could lead to the formation of an 'open window' within the nucleosomal structure, thereby increasing the accessibility of the DNA within the enhancer for binding of macromolecules involved in transcription. Of course, this does not exclude the possibility that sequences within the 72-bp repeat could be specifically recognized by some component of the transcription machinery, as was recently suggested by the stimulatory effect of the 72-bp repeat on *in vitro* transcription from heterologous promoter elements in the absence of evidence for chromatin reconstruction³⁴. One could speculate that the generation of an open chromatin structure and sequence-specific interaction between the 72-bp repeat and the transcription machinery act in concert to facilitate the activation of promoter elements embedded in the complex genome of higher eukaryotes.

The molecular mechanisms underlying the generation of altered chromatin structures over the 72-bp repeat are unknown. Several possibilities exist. *In vitro* reconstitution experiments showing that nucleosomes form less well on the ori region than on the rest of the SV40 genome suggest that some DNA sequences within the ori region cannot easily be folded around the histone octamer core^{35,36}. We note that it has been demonstrated recently that some sequences within and on the late side

of the 72-bp repeat can exist in the Z-DNA conformation *in vitro*³⁷ and that nucleosomes cannot be formed over Z-DNA³⁸. The alterations in chromatin structure may also be brought about by the binding of specific proteins, thereby inhibiting nucleosome formation. Interactions between enhancer sequences and specific cellular proteins have been suggested recently by studies demonstrating that the activity of some viral and cellular enhancers is cell- and/or species-specific (refs 39, 40 and references therein). Such proteins could be component(s) of the transcription machinery (see above) or belong to the class of proteins which specifically recognize Z-DNA⁴¹. Obviously, the identification of such putative proteins, the further development of *in vitro* systems, and the precise characterization of the sequences within the 72-bp repeat responsible for the enhancing activity and the generation of altered chromatin structures, are

required to elucidate the molecular mechanisms underlying these important biological functions.

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LETTERS TO NATURE

A new mine determination of the newtonian gravitational constant

S. C. Holding & G. J. Tuck

Physics Department, University of Queensland,
Brisbane 4067, Australia

Recent theoretical interest in the possibility of observing a non-newtonian component of gravity arises from the lead that such observations will give to unification theories¹. Absolute determinations of the newtonian constant G by geophysical methods, at mass separations several orders of magnitude greater than those available in the laboratory, provide information not obtainable in any other way. Stacey² drew attention to the fact that modern geophysical measurements were precise enough in principle to provide an important check on G and Stacey *et al.*³ revived the mine method first used by G. B. Airy. Subsequently Stacey analysed a set of overlapping sea-floor and sea-surface gravity survey data made available by the Exxon Oil Co., to avoid the problem of density uncertainty that beset the mine measurements, and Stacey and Tuck⁴ compared the results with values of G calculated from published mine and borehole data. The striking conclusion was that the values of G were all systematically high. Residual doubts, especially concerning the adequacy of density sampling and the possibility of bias by regional gravity anomalies, left the conclusion as a rather tentative one. We report here a new determination of G , which is also high, but in which the doubt about density is substantially reduced. This information makes it implausible to explain the high value of G in terms of an inadequate knowledge of density. The possible effect of regional gravity anomalies is not as securely discounted.

The measurements were made in the Hilton mine (24°34' S, 139°28' E), operated by Mount Isa Mines Ltd. We have made full use of the company's file of density data, from extensive coring of the area, and knowledge of geological structure by company staff. Over 2,300 core sample densities were used to establish the density pattern of the region. Gravity values were obtained at the surface and at 11 subsurface levels down to 993 m in stubs to one side of the P49 shaft (Fig. 1), using La Coste-Romberg meter G608. This was checked against the well calibrated G20 meter used previously and procedures for checking instrument drift, applying tidal corrections and so on, were the same as in the previously reported survey³. Data from the J53 shaft are not considered here as the density inhomogeneity immediately surrounding J53 and the shorter vertical extent of the shaft combine to give a much less accurate value of G . The equations for calculating G from the gravity profile and layer densities are given by Stacey *et al.*³ (and confirmed by Dahlen⁵), but a slight variation was required in the present case to take full account of the documented inhomogeneities.

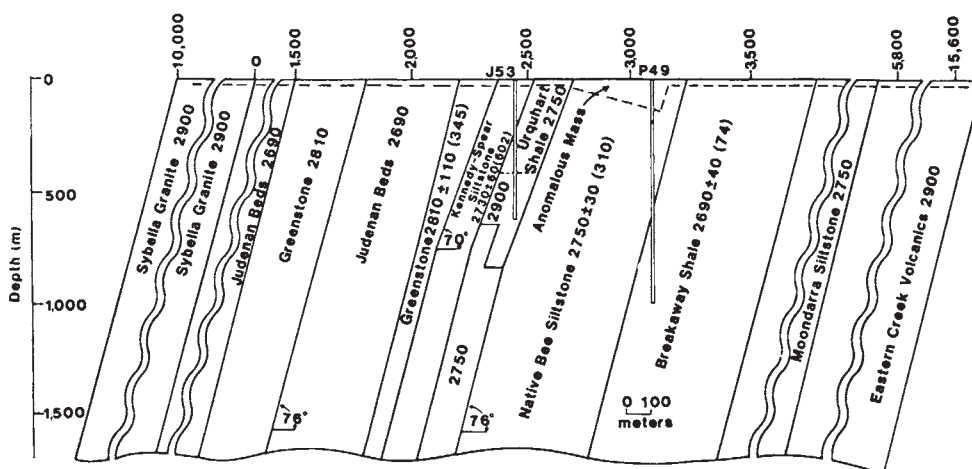
The method used by Stacey *et al.*³ was to average all density values at depths between gravity stations to obtain mean layer densities between those levels. Our method was first to assume a regional average density (2,750 kg m⁻³) and then to correct the individual gravity data for the localized departures from this average (using the laboratory value of G , 6,672 × 10⁻¹¹ m⁻³ kg⁻¹ s⁻²). This correction procedure was a fully three-dimensional one since we have a detailed picture of the geological structure and density of the region. The corrected gravity data were then used to calculate G (as in ref. 3). A small iterative adjustment was then applied by using the calculated value of G in repeating the corrections to the g values, but the effect on the estimate of G was not significant. Thus the density measurements were used to obtain representative densities of the iden-

Table 1 Vertical profile data for P49 shaft

Depth (m)	Gravity difference (10^{-3} m s^{-2})	Geological correction (10^{-6} m s^{-2})	Surface mass anomaly correction (10^{-6} m s^{-2})	$U(z)$ (10^{-3} m s^{-2})	$X(z)$ 10^6 kg m^{-2}	Gravity residuals (10^{-6} m s^{-2})
0	0	0	0	0	0	0
183.62	0.19382	1.45	-56.57	0.5669	0.5050	-4.74
243.45	0.23759	1.79	-52.78	0.7516	0.6695	-3.64
363.55	0.32471	2.55	-48.89	1.1224	0.9998	-5.73
483.55	0.41367	3.40	-46.71	1.4929	1.3298	-7.57
603.65	0.50187	4.29	-45.46	1.8637	1.6602	-11.05
723.50	0.59163	5.08	-44.46	2.2338	1.9898	-13.25
783.55	0.63614	5.43	-44.05	2.4192	2.1550	-14.90
813.55	0.65821	5.59	-43.86	2.5119	2.2375	-15.96
843.55	0.68131	5.65	-43.67	2.6045	2.3200	-16.09
963.53	0.77117	6.20	-43.02	2.9751	2.6501	-18.78
993.54	0.79386	6.38	-42.88	3.0678	2.7326	-19.28

Hilton mine: $g_0 = 9.786 \text{ m s}^{-2}$; $\phi_g = 20^\circ 34' \text{ S}$; height above sea level = 386 m. $X(z)$ values assume the reference density, $2,750 \text{ kg m}^{-3}$.

Fig. 1 Schematic cross-section of the rock formations of the Hilton mine (looking north). Structural trends are N-S, so that this cross-section is representative. Calculations assume the structure to extend to 4.5 km depth. Density values are given with standard deviations of individual samples about the means and the numbers of samples from each formation in parentheses. Gravity profiles were obtained for both shafts, but the greater homogeneity of rocks immediately surrounding the P49 shaft made this much more useful for calculating G .



tified geological formations instead of being simply averaged. The important difference between these procedures is that it avoids the bias of over-sampling of rocks that happen to be geologically interesting. Treatment of the density data for Urquhart shale required particular care, because this rock has some mineralization, but almost half of our density values were from it. The estimated mean density is a weighted average of the mineralized and unmineralized zones.

Special consideration is required for the density of the top 100 m or so of rock. This zone is weathered, porous and fractured, so that reliable and representative density data are impossible to obtain. We therefore chose to ignore the surface data point in calculating G and to consider the gravity gradient from 183 m (the first subsurface gravity station) to 993 m depth. This profile is unaffected by a uniform surface layer of unknown density. It is, however, influenced by inhomogeneity of the surface layer and a surface gravity survey was used to provide a correction for this. The surface survey was first corrected for the known deeper structure and then used to model a shallow mass to which we could attribute the residual gravity anomaly. This anomalous mass was then used to apply minor corrections to the subsurface g values; these corrections imply no knowledge of G , since they are based directly on comparisons of g .

All gravity data and corrections are listed in Table 1. The gravity values are corrected for instrumental drift and tides; topographic effects are insignificant. Excavation corrections are also included but these were made very small by mounting the gravity meter on a tripod near to the geometrical centre of a tunnel when a measurement is made. The first of the correction terms, as listed, was calculated assuming the laboratory value of G and was therefore re-weighted in the final calculation; the second is based on gravity measurements and so makes no assumption about G . $U(z)$ and $X(z)$ are depth-dependent parameters as defined by Stacey *et al.*³

The effectiveness of the density modelling is attested by the

small scatter of gravity data from the equation of Stacey *et al.*³ and consequently in the formal uncertainty of the calculated value of G :

$$G = (6.730 \pm 0.003) \times 10^{-11} \text{ m}^3 \text{ kg}^{-1} \text{ s}^{-2}$$

The quoted uncertainty represents one standard deviation and is clearly smaller than the possible systematic error in the measurement of density; it is simply an indication of the internal consistency in the data. This is also demonstrated by a plot of gravity residuals (Table 1, Fig. 2), that is the departures of the gravity data, relative to the surface value, from the values calculated assuming the laboratory value of G . We interpret the intercept on the graph of residuals to be primarily a result of poor density values for the surface layers, although in principle it could be used to infer the range of a supposed short range repulsive force additional to normal gravity. We cannot explain the poor fit of the data point at 183 m. If this is omitted then we obtain

$$G = (6.734 \pm 0.002) \times 10^{-11} \text{ m}^3 \text{ kg}^{-1} \text{ s}^{-2}$$

We are satisfied that inadequate knowledge of density must now be discounted as an explanation for the high values of G calculated here; this is probably true also for some of the earlier values and certainly for the marine measurement⁴. The remaining doubt arises from the possibility of a regional bias in the gravity gradient, as would be apparent if we could make a satisfactory direct measurement of the free-air gradient above the Earth's surface. The headframes at Hilton are not high enough to obtain satisfactory free-air gradient data. If we are to explain our observations in terms of conventional (newtonian) physics, we require a mass distribution at depth to give an anomalous vertical gradient of $-2.1 \text{ mgal km}^{-1}$ ($1 \text{ gal} = 10^{-2} \text{ m s}^{-2}$) (as in Fig. 2). For this gradient to be consistent to 10% over the top kilometre, as is apparent, the depth of the mass must be not less than 30 km. (It could be shallow and

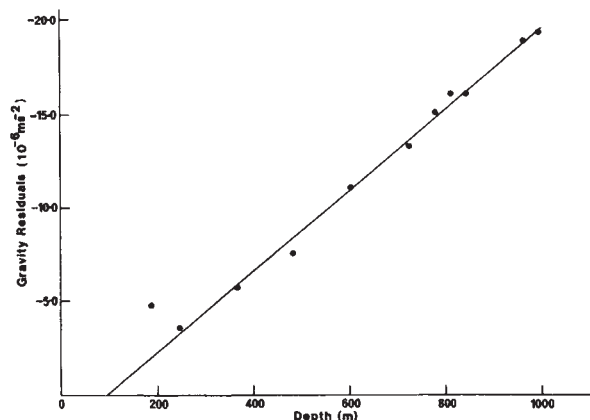


Fig. 2 Gravity residuals, calculated as differences between measured gravity at depth, relative to the surface, and values expected from the laboratory value of G .

laterally extended, but the calculations are similar.) Such a mass would cause a local gravity anomaly of about 30 mgal over a horizontal scale of about 30 km and would give a horizontal gradient greater than the locally observed one. However, the anomaly is comparable with the strongest of the observed gravity anomalies in the region⁶ so although it does not provide a convincing explanation for our observations, it introduces an element of doubt that we cannot dismiss with certainty.

Our value of G is the best controlled of the geophysical determinations, all of which are higher than the laboratory value⁴, and it falls into the middle of the range. Although we are not prepared, at this stage, to assert that a non-newtonian effect is clear and proven, the circumstantial evidence is very strong indeed. The need for a deep-sea measurement, as originally proposed², is now urgent.

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Hyperion: 13-day rotation from Voyager data

P. Thomas*, J. Veverka*, D. Wenkert†, G. E. Danielson† & Merton E. Davies‡

* Center for Radiophysics and Space Research, Cornell University, Ithaca, New York 14853, USA

† California Institute of Technology, Pasadena, California 91125, USA

‡ RAND Corporation, Santa Monica, California 90406, USA

The spin period and orientation of Hyperion have remained uncertain even with high-resolution Voyager images. We have now used low-resolution Voyager images taken over a two-month span to seek a rotational light-curve. The data indicate a spin period of 13 days, with the spin axis nearly parallel to the orbital plane.

Hyperion, an irregularly shaped satellite (maximum dimension about 350 km), orbits Saturn between Titan and Iapetus

once every 21.3 days. Because of the satellite's small size and large distance from Saturn, Peale¹ suggested that Hyperion's spin period may not be synchronous with its orbital period. The actual spin or rotation rate of Hyperion is of interest not only for dynamical reasons, but also in the context of understanding the albedo dichotomy on Iapetus^{2,3}. One popular explanation of Iapetus holds that dark dust spiralling into Saturn on retrograde orbits preferentially coats the leading hemisphere of Iapetus³, a scenario that implies that some of this dark dust is also intercepted by Hyperion. If Hyperion, like Iapetus, had a synchronous spin state one might expect to see large albedo differences between its trailing and leading hemispheres. Voyager images reveal no evidence of a hemispherical albedo dichotomy on Hyperion⁴.

Initial attempts to derive the rotation period of Hyperion from the highest resolution images obtained by Voyager 2 were not successful⁴. In this study we determine the rotation period by means of a light-curve derived from low-resolution Voyager images. We exclude the high-resolution, high phase-angle images obtained within a period of about one day, because during this period changes in the appearance of Hyperion reflected the rapid motion of the spacecraft rather than the intrinsic rotation of the satellite. Our analysis is restricted to nineteen images obtained through the clear filter ($\lambda_{\text{eff}} \sim 0.48 \mu\text{m}$) of the

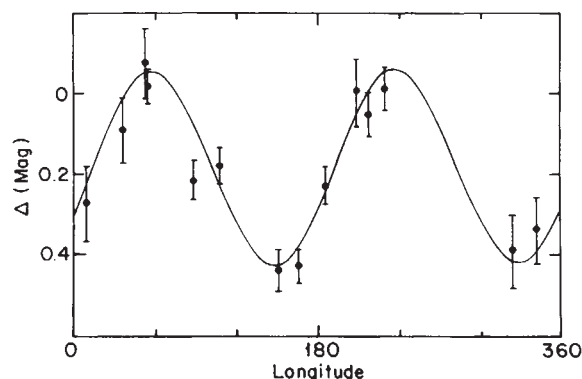


Fig. 1 Hyperion light-curve from Voyager 2 clear filter data, for a period of 13.1 days, rotation axis parallel to Hyperion's orbital plane. The observations are compared to a simple sine curve; error bars are estimated uncertainties due to phase function and photometric noise.

narrow angle cameras: five Voyager 1 images taken over 27 days from 26.5×10^6 km to 4.6×10^6 km, and fourteen Voyager 2 frames taken over 61 days from 59×10^6 to 1.8×10^6 km. The Voyager 1 data were from 14 October–10 November 1980 and Voyager 2 data 24 June–23 August 1981. For each image a disk-integrated brightness was obtained and scaled to a common distance. Corrections for phase effects were made starting from intrinsic phase coefficients of the surface material determined from the high resolution images at phase angles $\alpha = 25^\circ$ to 45° . The resulting average disk-integrated phase coefficients⁵ ranged from 0.019 to 0.020 mag per degree, and were used to scale the data to our reference frame (FDS 43323.29) obtained at $\alpha = 6.3^\circ$. The single image below a phase angle of 4.0° ($\alpha = 2.85^\circ$) was scaled using a phase coefficient of 0.03 mag per degree to allow for the possible presence of an opposition surge⁶. The calculated phase coefficients are consistent with those derived for other satellites of Saturn^{7,8}, and the precise value adopted has little influence on the light-curve analysis.

Uncertainties in the disk-integrated values used to construct the light-curve arise from two major sources. First, there is photometric noise given the very small scale of most of the images (1–5 pixels across). Second, for an object as irregular as Hyperion, corrections for differences in solar phase can only be made approximately, and corrections for variations in aspect must be ignored at this level of analysis. The resulting estimated uncertainties in the scaled brightness levels range from 4 to 10%.

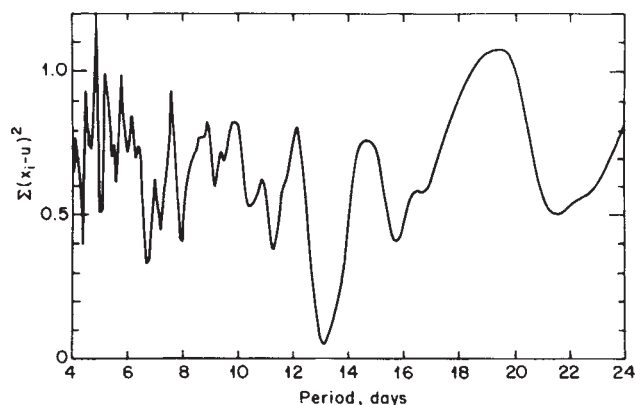


Fig. 2 Residuals of Voyager 2 observations (x_i) compared with a sine curve light-curve (u) as a function of rotation period. Differences in the orientation of the rotation axis produce only small differences in the residuals. The only good fits correspond to periods of 13.1 ± 0.3 days.

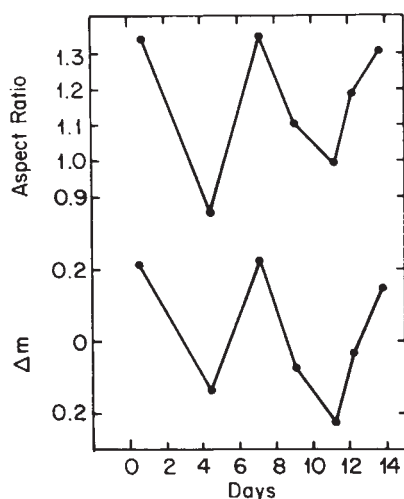


Fig. 3 The aspect of Hyperion and observed light-curve during the final 14 days of Voyager 2 approach data. The aspect ratio is the apparent dimension perpendicular to orbital plane divided by dimension parallel to orbital plane. The similarity of the two curves demonstrates that the light variations are primarily due to variations in the satellite's apparent shape, and that the spin axis is more nearly parallel to the orbital plane than perpendicular to it.

The available data indicate a spin period of 13 days (Figs 1 and 2). The result does not depend strongly on the assumed direction of the spin axis, because during each of the two sequences available the relative angular motions of the spacecraft and Hyperion were small. In Fig. 1 the Voyager 2 data are compared with a simple sine curve of period 13.1 days (the points cover five rotations of the satellite). By themselves, the five Voyager 1 points cannot be used to determine a period, but they are consistent (period and amplitude) with the light-curve shown in Fig. 1. Figure 2 shows the residuals between the Voyager 2 data and model sinusoidal lightcurves as a function of period. The only acceptable fits correspond to periods of 13.1 ± 0.3 days. If we use the estimates of uncertainties (see Fig. 1) a strong minimum occurs at the same period. Because the total uncertainties are not known, a formal χ^2 test is not appropriate.

We now turn to the possibility of determining the orientation of the satellite's spin axis. The last 14 days of Voyager 2 observations (seven images) obtained sufficient resolution to estimate the satellite's apparent shape. The longest dimension was found to be nearly perpendicular to Hyperion's orbital plane. The resulting aspect ratios (north-south dimension divided by east-west dimension) are shown in Fig. 3. It is evident that the light variations observed are due to changes in the apparent shape of the satellite as it rotates around its spin axis

and that the rotation axis must be nearly parallel to the orbital plane. The maximum Voyager 1 elongation (at maximum integrated brightness) was also nearly north-south and had an aspect ratio of 1.3.

Recently, Wisdom, *et al.*⁹, have suggested that Hyperion's spin state might be chaotic with substantial variations in both the period and the orientation of the spin axis on time scales of several orbital revolutions (period = 21.3 days). Our Voyager 2 data indicate a coherent 13-day spin period over 61 days, or nearly three orbital revolutions. Our data are also consistent with the view that Hyperion's spin period and spin axis orientation were similar 220 days earlier at the time of the Voyager 1 observations.

Our results show that Hyperion is definitely not in synchronous rotation. Consequently it cannot be used to test the hypothesis that dark dust spiralling inward on retrograde orbits causes the pronounced leading side/trailing side albedo asymmetry observed on the surface of Iapetus.

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Spatial structures generated by chemical reactions at interfaces

David Avnir & Michael Kagan

Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Far-from-equilibrium networks of physicochemical processes may exhibit structuring¹, in time (for example, chemical oscillations²), in space (stationary patterns³) or as a combination of both (chemical waves⁴). Much experimental effort has been invested during the past two decades in temporal and spatio-temporal phenomena^{2,4}; and except for periodic precipitation processes⁵ no general chemical system was available for a wide experimental study of the evolution of spatial structures. Recently, however, we have revealed a remarkably wide family of processes which produce spatial structures: chemical reactions at liquid interfaces. These include reactions at gas/liquid interfaces⁶, and photochemical reactions at liquid/air^{7,8} and liquid/liquid⁸ interfaces. We now report yet another important class: reactions at membrane surfaces, as shown in Fig. 1. Although hydrodynamic slow currents are apparent at a mature stage of the structure development, the actual mechanism leading to the initial structuring is as yet not clear. We show, however, that these structures are not a visualizing method of patterns caused by evaporative cooling^{9,10}: structures are formed also in the absence of such patterning and hydrodynamic currents start only after chemical structures are formed. Some of our observations suggest that chemical reaction is necessary for structure evolution, and that gravity apparently plays no role in this phenomenon.

The recognition that a much wider phenomenon lies behind Möckel's report¹¹ on structure formation in certain photo-



Fig. 1 Chemical structures at membrane surfaces. *a*, Patterns of iodine–starch complex resulting from the diffusion of bromine into KI/starch. *b*, Patterns of $\text{KFe}(\text{NCS})_4$ in the reaction of FeCl_3 with KSCN .

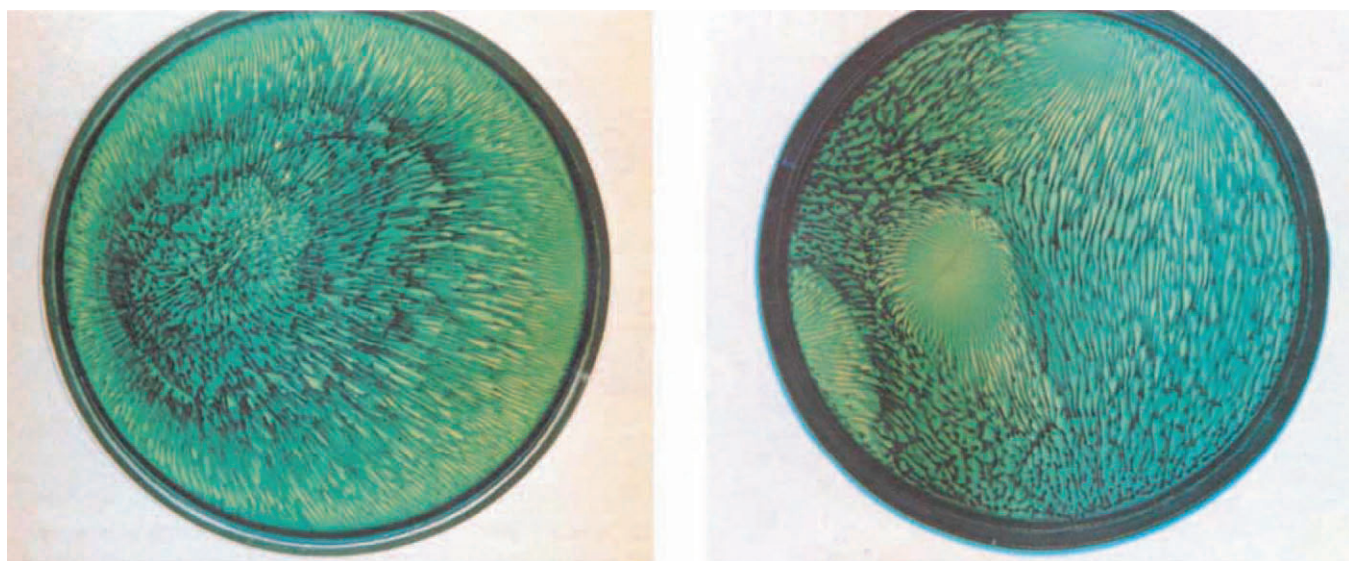


Fig. 2 Structures of Turnbull's blue formed by photoreduction of ferricyanide in a sealed Petri dish. *a*, With an air gap between the liquid surface and the glass seal. *b*, No air gap.

chemical reactions, has been developed through recent exploratory research. First, we reported that almost any photo-halogen cleavage, followed by a thermal halogen reaction produced spectacular far-from-equilibrium structures^{7,8}. It was then shown by Micheau *et al.*^{9,10}, and later by us¹² and by others^{13,14}, that light-energy can be used quite generally to drive reacting systems away from equilibrium thus forming structures of photo-products. We were able then to substitute energy influx through the liquid interface, by matter influx, and showed that the reaction between a diffusing gas and a reactant in solution proceeds in a nonhomogeneous structured way⁶. An interface which is of special importance because of its role in biology, is that between two reacting solutions separated by a membrane. We report now that dissipative¹ structures are formed near the surface of a dialysis membrane (Spectropore-2, molecular cutoff 12,000) when one species diffuses through the membrane and reacts on the other side; the phenomenon is general from the chemical point of view (Table 1). All types of structure-formation systems described above share in common the sequence of

events shown in Fig. 3. The membrane reactions were carried out as follows: A Perspex cell, having two compartments 5 cm in height, separated by the membrane, was filled up with the two solutions, one in each of the compartments. In all reactions (see Table 1 and Fig. 1) a solution of a small species is separated from a dilute solution of a larger substrate, so that diffusion is virtually unidirectional. The coloured product is also a large molecule. The following observations indicated in Table 1 should be especially noted: (1) completely unrelated reactions produce similar patterns. As was found in our previous systems, the nature of the chemical reaction is of secondary importance. In fact, this generality raises the question of how this observation has been overlooked so far. We have referred already to this question when we described the gas/liquid structures⁶, and suggested that the observation has been overlooked firstly because of the relative lack of awareness in chemistry to the importance of macroscopic structure-building processes; and secondly, because reactions are usually carried out under stirring: any structure formed would have been destroyed. (2) The

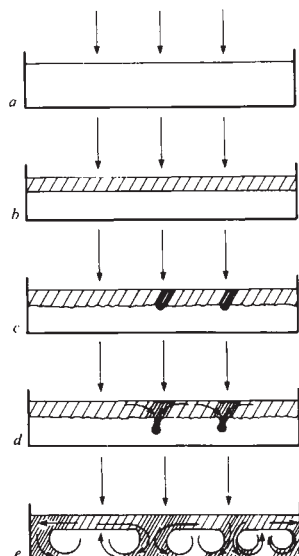


Fig. 3 *a*, Before reaction starts, whether photolytic, gas/liquid or liquid/liquid, the reactants solution is homogeneous and at rest. *b*, The reaction starts over the entire interface. *c*, After about 60 s lines of higher concentration begin to appear. *d*, The product descends along the pattern lines. If the interface is at the bottom the product *ascends*. *e*, If the solution is shallow enough (about 15 mm) the descending product forms hydrodynamic rolls. The solution eventually becomes homogenous again (not shown).

I_2 /starch structures were produced not only through a membrane, but also by photooxidizing an I^- /starch solution^{7,11} (Fig. 4, see below), and by I_2 vapours diffusing through starch solution interface⁶. The generality of the nature of the interface in this phenomenon is thus shown. (3) Comparison of reactions 2 and 3 (Table 1) suggests that gravity is not playing a deciding role in the overall mechanism. This is also suggested when photochemical structure-forming systems are irradiated from the bottom through a glass/liquid interface (refs 10, 12 and the Fe(III) photoreduction described below). (4) Note that in the cases where diffusion was not accompanied by a chemical reaction, structures did not form (reactions 10 and 11 in Table 1).

The apparent similarity between the patterns described and bulk hydrodynamic patterns¹⁵ suggests the possibility that the patterns are colour tracing preexisting evaporative hydrodynamic currents (prepatterns)^{9,10}. However, we present evidence that chemical patterns evolve in the absence of any evaporative prepatterns.

A number of experiments have been carried out with the photoreduction of Fe(III) to Fe(II) (ref. 16). A Petri dish filled with 5–10 mm of an aqueous solution of 0.2% oxalic acid

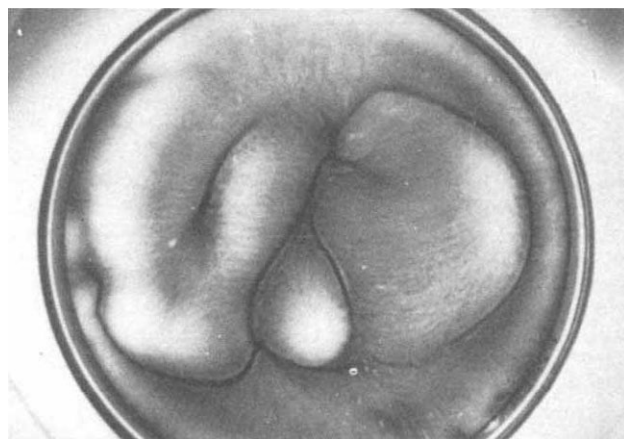


Fig. 4 An iodine-starch pattern formed by photolysis of KI/chloralhydrate/starch at 254 nm.

dihydrate, 0.2% potassium ferricyanide and 0.1% ferric chloride hexahydrate (solution (1)) was irradiated from above at 360 nm (4×15 W lamps). The ferrous ions produced in this reaction form $Fe_3[Fe(CN)_6]_2$ (Turnbull's blue). This photochemical reaction, which produces patterns, was found to be experimentally more convenient than our previously reported photochemical reactions^{7,8}. Evaporative currents in this system can easily be followed by sprinkling aluminium dust and illuminating from the side with a laser. To avoid the evaporative patterns, the Petri dish was sealed tightly with a glass cover. As expected^{15,17}, the evaporation patterns disappeared and no other convections could be detected. The liquid/air interface was then irradiated through the seal: beautiful blue structures were formed (Fig. 2a). The sequence described in Fig. 3 was very clear: first, the surface was homogeneously charged with the photoproduct (again, evaporation would clearly be seen at this stage); after about a minute, patterns started to grow; and finally bulk movements appeared when the photoproduct started to descend from the line-patterns at the surface, that is, the onset of currents is a later stage, and not a prerequisite, in this phenomenon.

In a second experiment on this photochemical system 25% methanol was added in order to enhance the evaporative patterns. Such binary systems are known to have pronounced patterning^{15,18}. The initial experiment was repeated and compared to an open Petri dish irradiation. Although we cannot rule out the possibility that the produced patterns in the open vessel coincide with the evaporative patterns¹⁰, it is our observation that the evaporation in fact interferes with an ordered still growth of the patterns. Furthermore, we found it extremely difficult to get chemical patterns in open vessels containing

Table 1 Structure formation at membrane surfaces

Solution* above membrane	Solution* below membrane	Reaction type	Colour of structure
0.01% Iodine	0.5% Starch	Complexation	Blue
0.1% Bromine	0.5% Starch/1% KI	Oxidation followed by complexation	Blue
0.5% Starch/1% KI	0.1% Bromine		Blue
0.01 M HCl	0.02% Methyl orange	Protonation	Red
0.01 M KOH	0.02% Phenolphthalein	De-protonation	Pink
0.5% Acetaldehyde	20% Morpholine	Oxidation	Blue
	5% Sodium nitroprusside		
1% $FeCl_3$	0.1% $K_4Fe(CN)_6$	Complexation	Blue
1% $FeCl_3$	0.1% Tannic acid	Complexation	Grey
1% $FeCl_3$	0.1% KSCN	Complexation	Red
0.01% Methylene blue	Water		No structure (blue)
Water	0.02% Methyl orange/0.01 M HCl		No structure (red)

* All solutions in water.

liquids of very high vapour pressure such as methylene dichloride⁸.

In a third experiment the Petri dish was filled with solution (1) to the rim, and then covered with a 3 mm sheet of glass, that is, the liquid/air interface was replaced by a liquid/glass interface. Obviously there is no evaporation in this system, yet structures developed in the usual way (Fig. 2b). Similarly, structures were obtained when the Petri dish (either closed or open) was irradiated from below. These observations seem to exclude a Marangoni-type effect¹⁴, which requires a free surface¹⁹. Structures appeared also upon irradiation of a very shallow layer, obtained by 'sandwiching' solution (1) between two glass plates separated by 0.95 mm. Pattern formation upon irradiation of a 1 mm layer was reported by Micheau *et al.* in an open system and attributed to preexisting currents^{9,10}. However, a careful observation under a microscope of the sequence of events on irradiating one drop of (1) (~3 mm in diameter, less than 1 mm in height) revealed again the stages of Fig. 3, namely: first pattern formation at the surface and only then slow hydrodynamic currents.

Other evidence shows, as we have reported already⁸, that irradiation of the following liquid interfaces, protected from evaporation by another 4 cm liquid layer, produced structures: diphenylamine/ CCl_4 covered by water; diphenylamine sulphate/ $\text{KBr}/\text{H}_2\text{O}$, covered by cyclohexane; 4-aminophenol/ $\text{HCl}/\text{KBr}/\text{H}_2\text{O}$, covered by paraffin oil. The membrane experiments described in this paper again exclude the necessity for evaporative pre patterning for the chemical structures formation: the membrane is covered with a 5 cm layer, and protected at the sides by 2 cm Perspex walls.

Reactions 10, 11 in the Table (dye diffusion only) demonstrate that diffusion by itself is probably insufficient in accounting for the phenomenon, and that coupling with a chemical reaction is necessary. The quantitative evaluation and comparison of one dissipative structure to the other is a complicated, still uncompleted task that has been undertaken by us¹² and by others¹⁷. However, even at this early stage it seems to us that in many cases (for example, Fig. 4) one can tell qualitatively that the shapes obtained are in fact quite different from usual patterns seen in thermal convections¹⁵.

Models and theories of far-from-equilibrium processes have predicted the feasibility of the formation of interfacial structures²⁰, yet to the best of our knowledge, the phenomenon we describe is the first wide family of chemical processes forming such structures. Quantitative parametrizations and simulations in the light of such models, and a study of an interesting scaling behaviour of the patterns¹², found during the microscopic experiment described above, are in progress.

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A new chemical oscillator containing neither metal nor oxyhalogen ions

Maria Burger* & Richard J. Field

Department of Chemistry, University of Montana, Missoula, Montana 59812, USA

Essentially all known inorganic chemical oscillators¹ contain a metal ion²⁻⁴ and/or an oxyhalogen ion, in particular, iodate ion³, bromate ion^{2,5} or chlorite ion⁶. We report here a new chemical oscillator that is based on sulphur chemistry and contains neither a metal ion nor an oxyhalogen ion. The oscillator does involve elemental oxygen, and in this way it may be related to an oscillating reaction recently discovered by Jensen⁷. The discovery of new chemical oscillators which are fundamentally different from those previously known is of great importance as it supplies new testing grounds for general theories of dynamic behaviour⁸ as well as further information concerning the exact mechanistic requirements for chemical oscillators to occur.

The chemical oscillator is composed of sulphide ion, sulphite ion, methylene blue (which serves as a catalyst), and dissolved oxygen. During the reaction, the methylene blue catalyst oscillates between its oxidized, coloured form (MB^+) and its reduced, leuco form (MBH). Figure 1 shows a typical oscillatory trace of the absorbance at 668 nm due to MB^+ . Sharp pulses of oxidation are observed in which ~40% of the MBH is converted to MB^+ .

The reaction is run in an aqueous medium of pH 12-13 and in a continuous-flow, stirred tank reactor (CSTR). It is the only known example of chemical oscillations in a basic solution. The three feedstreams contain, respectively, MB^+ , Na_2S , Na_2SO_3 and O_2 at the appropriate pH. The sulphide ion is essentially completely tied up as HS^- at pH 12-13. Sulphite ion expands the range of feedstream reactant concentrations and reactor residence times for which oscillations are found. However, it is apparently not a major reactant as oscillations appear in its absence. Figure 2 shows the region in $[\text{S}_2^{2-}]$, $[\text{SO}_3^{2-}]$ constraint-space for which oscillations occur at a particular flow rate, oxygen concentration and methylene blue concentration. The experiment is run in CSTR mode both to maintain the chemical system far from thermodynamic equilibrium, a necessity for sustained chemical oscillations⁹, and to keep the reactant concentrations, especially that of dissolved oxygen, in the oscillatory range. The dynamics of the flow does couple with the chemical dynamics. However, regular but irreproducible oscillations have been observed (B. Miewald and R.J.F., unpublished data) when the experiment is run in a batch reactor.

The quantitative behaviour of the reaction is rather sensitive to impurities in the Na_2S used, mainly sulphite ion, polysulphide ion and thiosulphate ion. However, the reaction is quite reproducible when care is taken to monitor and control these impurities quantitatively.

Oscillations appear only in a rather small range of flow rates for a particular set of feedstream concentrations, and the oscillatory region is quite dependent on these concentrations. Typically, the steady-state $[\text{MB}^+]$ is very close to zero at flow rates below the oscillatory region while at flow rates higher than the oscillatory range it is substantially higher, approximating the median $[\text{MB}^+]$ during an oscillation. Approach to steady states may be very slow both above and below the oscillatory range. Bistability is a common feature of chemical oscillators run in CSTR mode¹⁰. However, we have not yet observed bistability in this system. The search continues.

* Permanent address: Institute of Inorganic and Analytical Chemistry, L. Eötvös University, Múzeum KRT 4/B, Budapest, Hungary.

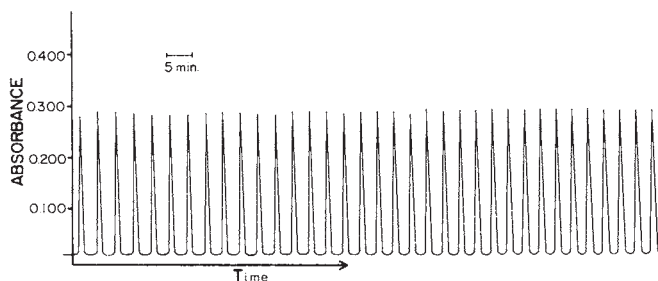


Fig. 1 Oscillations in the concentration of MB^+ as observed by measuring the absorbance at 668 nm. Conditions: $[\text{MB}^+]_0 = 3 \times 10^{-6} \text{ M}$; $[\text{SO}_3^{2-}]_0 = 1.2 \times 10^{-3} \text{ M}$; $[\text{S}^{2-}]_0 = 4.1 \times 10^{-2} \text{ M}$; $[\text{HO}^-]_0 = 5 \times 10^{-2} \text{ M}$; $[\text{O}_2]_0 = 1.5 \times 10^{-4} \text{ M}$. Temperature, $20 \pm 0.1^\circ \text{C}$. $1/(\text{residence time}) = 1.5 \times 10^{-3} \text{ s}^{-1}$.

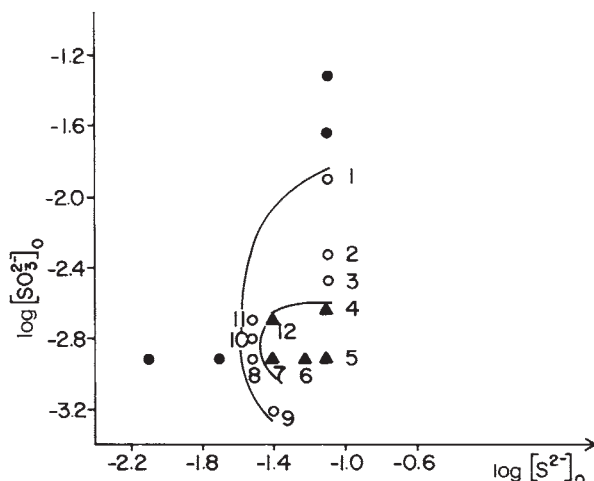


Fig. 2 $[\text{S}^{2-}]_0$ versus $[\text{SO}_3^{2-}]_0$ constraint-space diagram. Conditions: $[\text{MB}^+]_0 = 3.0 \times 10^{-6} \text{ M}$; $[\text{HO}^-]_0 = 5 \times 10^{-2} \text{ M}$; $[\text{O}_2]_0 = 1.5 \times 10^{-4} \text{ M}$. Temperature, $20 \pm 0.1^\circ \text{C}$. $\circ$, Transient oscillations as the reciprocal residence times noted below are approached: $\bullet$, stable steady state; $\blacktriangle$, sustained oscillations. The reciprocal residence times are: (1) $1.9 \times 10^{-3} \text{ s}^{-1}$; (2) $3.5 \times 10^{-3} \text{ s}^{-1}$ and $3.1 \times 10^{-3} \text{ s}^{-1}$; (3) $3.1 \times 10^{-3} \text{ s}^{-1}$; (4) $2.3 \times 10^{-3} \text{ s}^{-1}$ and $1.9 \times 10^{-3} \text{ s}^{-1}$; (5) $2.0 \times 10^{-3} \text{ s}^{-1}$; (6) $2.0 \times 10^{-3} \text{ s}^{-1}$; (7) $1.6 \times 10^{-3} \text{ s}^{-1}$ and $1.3 \times 10^{-3} \text{ s}^{-1}$; (8) $1.3 \times 10^{-3} \text{ s}^{-1}$ and $1.0 \times 10^{-3} \text{ s}^{-1}$; (9) $1.5 \times 10^{-3} \text{ s}^{-1}$; (10) $1.5 \times 10^{-3} \text{ s}^{-1}$; (11) $1.3 \times 10^{-3} \text{ s}^{-1}$; (12) $1.3 \times 10^{-3} \text{ s}^{-1}$.

The net stoichiometric chemical reaction which drives the oscillations is apparently reaction (1).



The elemental sulphur may polymerize according to reaction (2),



or be removed from the system by sulphite ion according to reaction (3).



Substantial amounts of elemental sulphur may precipitate in experiments where $[\text{SO}_3^{2-}]$ is low. However, this does not strongly affect the oscillations. Indeed, elemental sulphur may be added to the feedstreams with only minor effect.

This new oscillator was intentionally designed by the expedient of coupling an apparently autocatalytic reaction with a return mechanism. Reaction (4), the reduction of MB^+ by HS^- , is the apparently autocatalytic reaction¹¹.



However, we have now found (unpublished data) that the reaction is not genuinely autocatalytic. It is instead inhibited by oxygen, which must be removed before the reaction can begin. A similar inhibition has been observed in the reduction MB^+

by sulphhydryl compounds¹² such as cysteine. We have been unable to observe oscillations in the present system when sulphide ion is replaced by cysteine.

The return step is the oxidation of MBH by O_2 . There is evidence¹³ that this reaction actually is autocatalytic.

An explanation of the mechanism of this oscillator must await further detailed information on the component reactions. This work is underway.

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Evidence for stabilization of the Pilbara Block, Australia

T. S. Blake

Department of Geology, University of Western Australia, Nedlands, 6009 Western Australia

The stabilization of continental crust to form cratons represents one of the major features of Earth history¹. Major structural, geochemical^{2,3} and, to a lesser extent, sedimentological changes⁴ associated with the development of stable crustal blocks are recorded mainly around 2,500 Myr ago¹. This change, however, appears to have been globally diachronous and cratonic sequences as old as 3,100 Myr are recorded⁵. I now present the results of a recent regional stratigraphical and sedimentological study on the ~2770 Myr old lower Fortescue Group^{6,7} (previously classified lithologically as Proterozoic⁸, but following the recent recommendation of a 2,500-Myr Archaean/Proterozoic chronological boundary⁹ is referred to as Archaean) in the Pilbara Block¹⁰, Western Australia. A major stabilization of the underlying granitoid-greenstone terrain appears to have pre-dated Fortescue basin formation. This event, which available geochronology suggests was relatively rapid, marked a major changing point in the tectonic evolution of the Pilbara Block.

The lower Fortescue Group (Fig. 1) comprises shallow-dipping, very low-grade¹¹ flood basalts, clastic sediments and tuffs which rest unconformably on a deformed 3,500–2,850 Myr old granitoid-greenstone terrain¹², the Pilbara Craton¹⁰. The age of the Fortescue Group is constrained by: (1) $2,847 \pm 34$ Myr, Pb–Pb whole rock age from a post-tectonic granitoid in the underlying Archaean craton (U.W.A., unpublished data, revises previous Rb–Sr ages^{13,14}). (2) $2,768 \pm 16$ Myr, U–Pb zircon age from a porphyry with the lower Fortescue Group (R. T. Pidgeon, personal communication) that was originally considered intrusive¹⁵ but is now reinterpreted as having an erosive upper contact (upper Unit 2, East Pilbara Basin, Fig. 2). (3) $2,490 \pm 20$ Myr and $2,470 \pm 30$ Myr, U–Pb zircon ages from the overlying Hamersley Group¹⁶. The basal unconformity

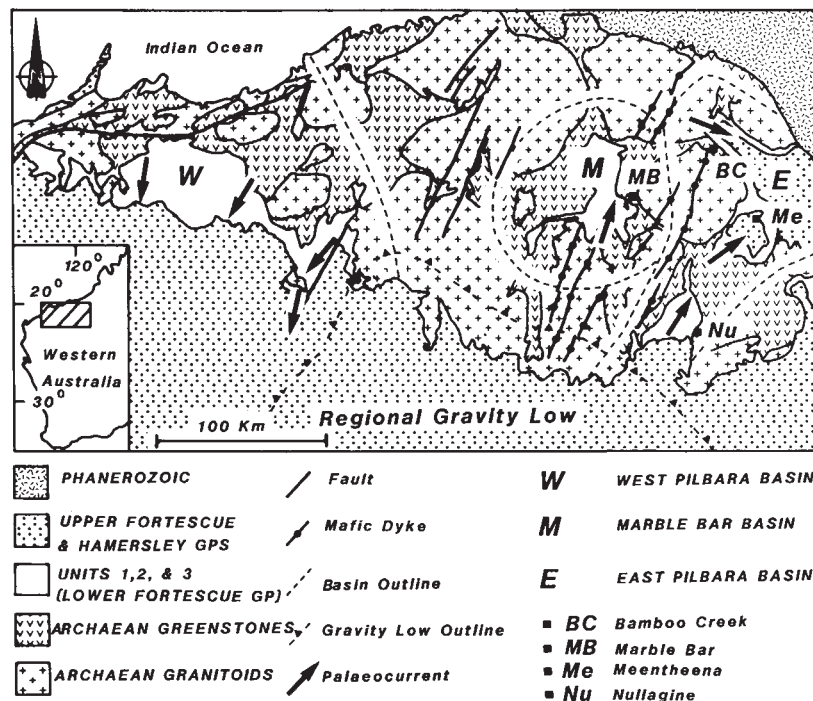


Fig. 1 Location of study area, outline of early Fortescue Group basins and unit 3 palaeocurrents.

(Fig. 2) therefore probably represents a maximum hiatus of ~130 Myr, although the contact between the dated Archaean post-tectonic granitoid and the lower Fortescue Group is not seen.

Three discrete lower Fortescue basins have been recognized, the West Pilbara, Marble Bar and East Pilbara Basins (Fig. 1). These are lithostratigraphically correlated using regional intra-Fortescue unconformities which divide the revised stratigraphy into three major units (Fig. 2). These units sequentially reflect a change in tectonic controls on sedimentation.

Unit 1 comprises a discontinuous basal clastic sedimentary horizon commonly <30 m thick, overlain by up to 2,000 m of subaerial to shallow-water flood basalt. In the Marble Bar Basin, however, up to 500 m of fluvial sandstone and conglomerate exists beneath the basalt. The local derivation of sediment (from Archaean granitoids and greenstones) and converging fluvial palaeocurrents suggest deposition in an enclosed basin centred over greenstones (Fig. 3). Deformation preceded unit 2 sedimentation, resulting in dips of 5–20° towards the centre of the earlier sedimentary basin. Local close to tight folding (dips 50–60°, in places vertical) formed a sinuous central belt of upright, shallow-plunging structures (Fig. 3). This style of deformation is similar to the main upright deformation affecting the underlying greenstone belts which has been assigned to diapiric movements on surrounding granitoids¹⁷.

Unit 2, unconformably overlying Archaean greenstones and unit 1, exists only in the East Pilbara and Marble Bar Basins (Fig. 2). In the East Pilbara Basin it comprises a poorly exposed fluvial and lacustrine sequence overlain by an intrusive and extrusive acid porphyry complex. In the Marble Bar Basin, unit 2 mainly comprises subaerial to shallow-water basaltic lavas with complex interdigitations of sediments near the base. Most of the sediments, which are of granitoid provenance, are preserved as three, up to 80 m thick, upward-coarsening sequences (shale/silt to sandstone/pebble conglomerate) deposited axially in an elongate basin ~15 km wide and at least 40 km long. Initial fluvial sedimentary input was from the north with a later influx from the south-east. The coarsening upward sequences probably resulted from fluvial progradation into a lacustrine environment. The cyclicity of sedimentation suggests an active fault control, although the exact positions of these faults are unclear. Most observed fractures were generated by major post-unit 2 movements. The style of sedimentation, in possibly fault-controlled belts with laterally persistent stratigraphy and a more

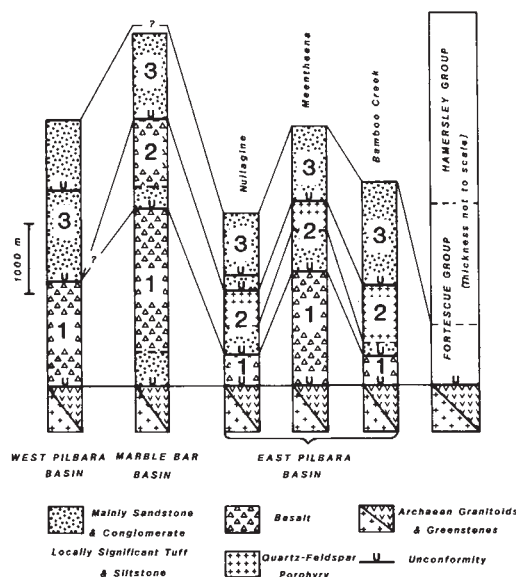


Fig. 2 Stratigraphical position of units 1, 2 and 3 and revised stratigraphy (cumulative maximum thicknesses).

uniform provenance, is in marked contrast to the sedimentation in unit 1, although in both units sediment accumulated over greenstone belts.

Unit 3 is dominated by granitoid derived sandstones with pebble conglomerates and minor coarser conglomerate, except in the West Pilbara Basin where it is mainly composed of epiclastic volcanic sediments. Fluvial palaeocurrents (Fig. 1) indicate axial sediment dispersal in linear troughs centred over greenstone belts. These basins were ~20 km wide and at least 100 km long (satellite imagery suggests the main north–north-east trending East Pilbara Basin structure may have been >250 km long). Fault-controlled local marginal alluvial fan systems (up to 1 km thick) suggest deposition in part in elongated graben structures. Active basin margin faulting apparently became less important towards the top of unit 3, sedimentation patterns remained linear and parallel to the underlying troughs, although basins became significantly wider and marginal sedimentation overlapped earlier growth faults.

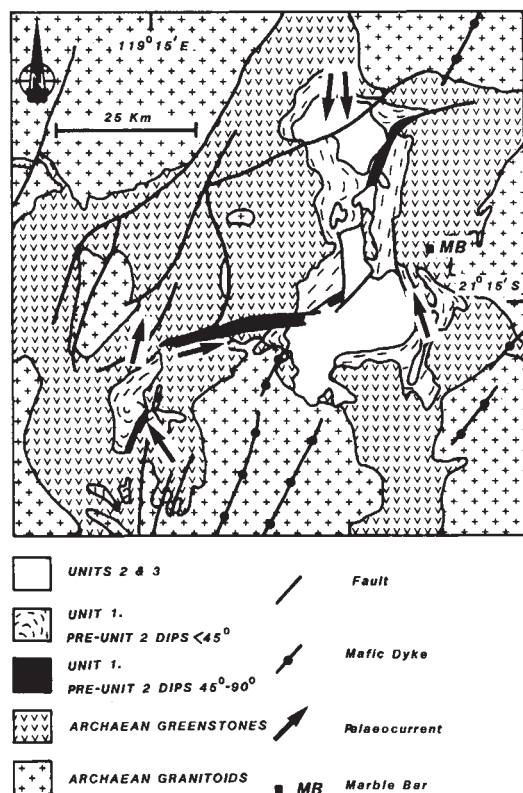


Fig. 3 Marble Bar Basin, unit 1 palaeocurrents and deformation belt.

Unit 3 palaeocurrent data (Fig. 1) indicate a northward palaeoslope in the Marble Bar and East Pilbara Basins and a southward palaeoslope in the West Pilbara Basin. Unit 3 was not deposited between these two regions where units higher in the Fortescue Group overlap the Archaean basement. Bouguer anomalies¹⁸ show a regional gravity low in this area, probably reflecting a preponderance of low density granitoids in the underlying Archaean craton. Such an area would have been prone to gravitational uplift and appears to have been a topographic high during deposition of the lower Fortescue Group¹⁹.

In the East Pilbara large (up to 100 km long and 150 m wide) north-north-east trending undeformed mafic dykes cross-cut Archaean granitoids and greenstones. The relationship between these and the lower Fortescue Group is generally inconclusive, although the latest phases clearly post-date unit 2 and probably unit 3.

The lower Fortescue Group sedimentary stratigraphy reflects an increasing fracture control on sedimentation culminating in >250 km long, mostly north-north-east trending, fractures during unit 3. Initially, sedimentary basin geometry and deformation may have been locally controlled by minor intra-cratonic diapiric movements on Archaean granitoids. The effusion of extensive flood basalts during unit 1 (now preferentially preserved over greenstone belts), however, suggests that basins were initiated under a tensional regime. Fracturing became less important during the upper part of unit 3 and a more regional subsidence is apparent, resulting in the probable unification of all three initial basins during upper Fortescue and Hamersley times. The north-north-east trending mafic dykes (possibly feeders to some of the basalts) cross-cut the Archaean craton irrespective of lithology or structural trends, exploiting the same fracture system that controlled sedimentary basins. This disregard for Archaean crustal trends and the inferred tapping of deep magma sources suggest failure of the lithosphere rather than the crust.

The initiation of Fortescue Group accumulation appears to have been caused by the superimposition of a regional tensional regime on a stabilized Archaean granitoid-greenstone terrain,

although inferred minor diapiric movements suggest that some internal mobility remained. A significant lithospheric thickening and associated stabilization during the 130 Myr between the latest Archaean plutonism and initial Fortescue deposition would account for a change from crustal to lithospheric dominated tectonics. Although the causes of such an event remain speculative, a localized rapid decrease in heat flow^{20,21} would have such an effect. In this context, it is noteworthy that the proposed period of cratonization (≤ 130 Myr) is of the order of the present thermal time constant²². A rapid local decline in heat flow and subsequent lithospheric thickening and stabilization, as opposed to a secular global decrease, accords with the worldwide diachronous record (3,100–2,500 Myr) of continental cratonization.

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Cambrian and Ordovician conodonts from the Robertson Bay Group, Antarctica and their tectonic significance

Clive F. Burrett & Robert H. Findlay

Geology Department, University of Tasmania, GPO Box 252C, Hobart, Tasmania, Australia 7001

The Robertson Bay Group is a thick (>3,000 m) sequence of turbidites deposited in one or more submarine-fan complexes^{1–3}. It is exposed throughout the eastern half of North Victoria Land and has been assigned variously to the Upper Precambrian⁴ (on the basis of acritarchs and lack of body fossils), or to the Lower Cambrian⁵ or Cambrian² (partly on the basis of trace fossils). We report here Upper Cambrian and Lower Ordovician conodonts in a limestone block within rocks mapped^{3,6} as Robertson Bay Group in the southeastern part of North Victoria Land. This discovery necessitates a reappraisal of tectonic reconstructions of the pre-Mesozoic units of south-east Australia, New Zealand and Antarctica^{7–17}. A reconstruction is proposed which includes correlation of the Lower Ordovician Greenland Group, New Zealand and the Upper Cambrian–Lower Ordovician Wierah Formation of southern Tasmania.

During the third German North Victoria Land Expedition (GANOVEX III) in the southern summer 1982–83, 4 kg

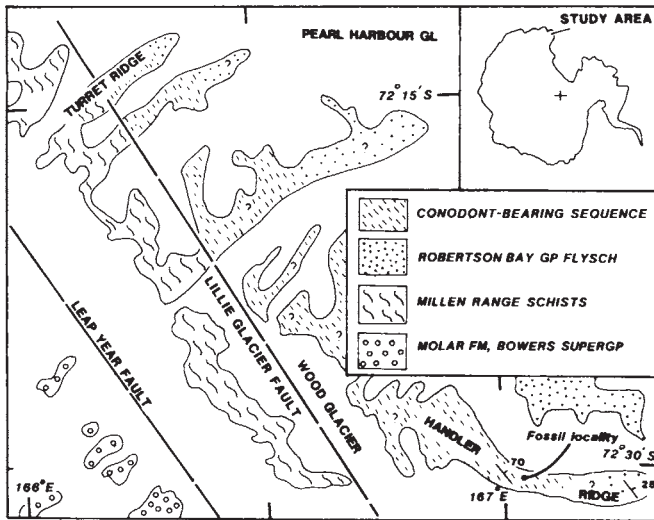


Fig. 1 Geological map of southeast part of North Victoria Land, Antarctica. East-west distance across map is 46 km.

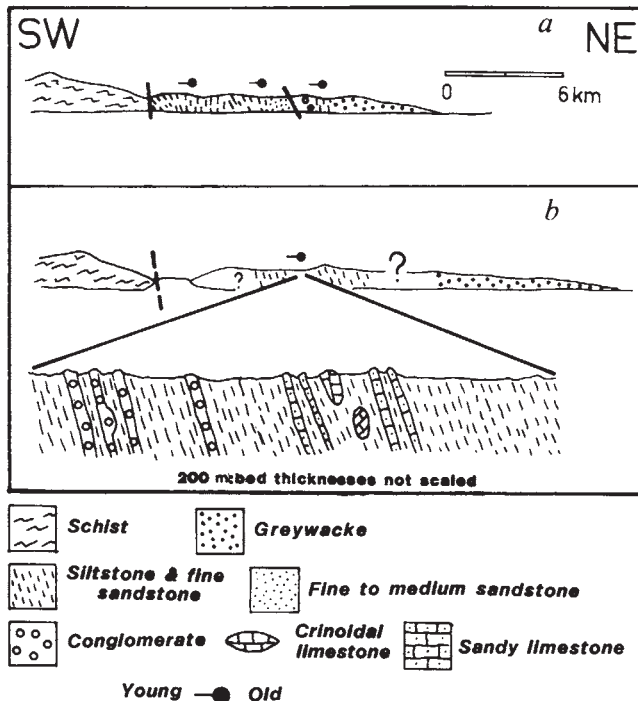


Fig. 2 Diagrammatic cross sections across a, Turret Ridge, and b, Handler Ridge.

of crinoidal intrabiosparite were obtained at Handler Ridge (Fig. 1) from a 1×0.5 m carbonate block within a sequence dominated by strongly cleaved, fine-grained, siliciclastics interbedded with subordinate, thin, matrix-supported siliceous conglomerate with pebbles of vein-quartz, limestone, chert and fine-grained sandstone and mudstone (Fig. 2). One fist-sized carbonate clast adjacent to the block has yielded a trilobite fauna currently being studied by T. Wright and colleagues in the United States. A similar sequence is exposed on Turret Ridge, some 30 km northwest (Fig. 1). Unfortunately, logistic problems prevented detailed stratigraphic logging of the section but the sequence appears to grade northeast into typical Robertson Bay Group turbidites.

Folds at the fossil locality plunge steeply (60–90°) in contrast to the generally shallowly plunging (0–25°) structures found in the Robertson Bay Group at the east end of Handler Ridge

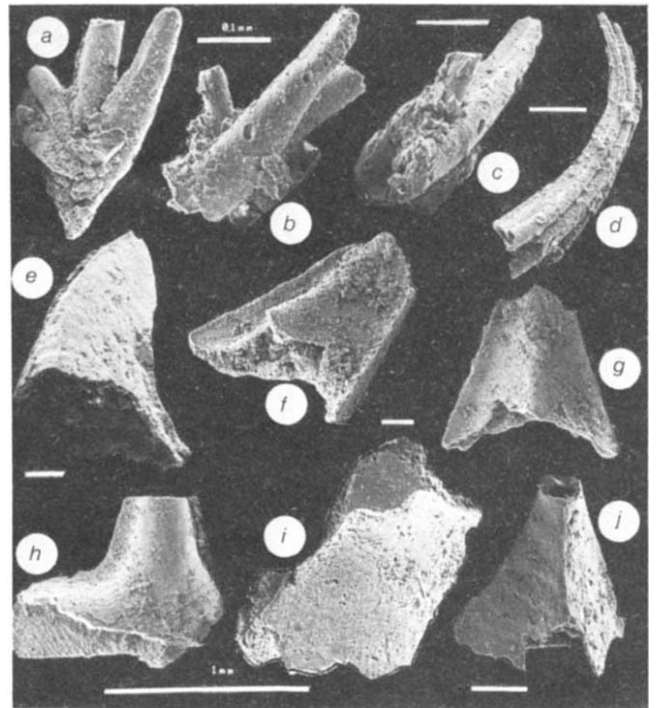


Fig. 3 Conodonts from limestone block within Robertson Bay Group, Handler Ridge. a–c, Fused assemblage of four elements of *Proconodontus posterocostatus* Miller 1980; d, '*Prooneotodus*' *tenuis* (Müller 1959); e, *Proconodontus* aff. *muelleri* Müller 1969; f–g, *Prosagittodontus* aff. *dunderbergiae* Müller 1959; h–i, *Iapetognathus* sp.; j, *Westergaardodina* aff. *bicuspidata* Müller 1959. Scale bar, 0.1 mm except for h–i, 1 mm.

and predominant^{18,19} elsewhere in the Robertson Bay Group. However, this contrast is not diagnostic of a tectonic distinction between the rocks at the fossil locality and the more typical Robertson Bay Group rocks to the east, as the folds throughout Handler Ridge possess the same northwest-trending axial plane cleavage. This cleavage occurs throughout the Robertson Bay Group, and is axial plane to primary non-cylindrical folds in Robertson Bay Group rocks on the northeast side of Pearl Harbour Glacier¹⁹ and elsewhere¹⁸.

On acetic acid dissolution, the limestone samples yielded a fauna of paraconodonts, conodontophorids, inarticulate brachiopods and phosphatocopine ostracods. Also present are poorly preserved, indeterminate, asaphid trilobites.

The conodont fauna includes '*Prooneotodus*' *tenuis* (Müller 1959), *Prosagittodontus* aff. *dunderbergiae* (Müller 1959), *Furnishina* spp., *Problematoconites* sp., *Westergaardodina* aff. *bicuspidata* Müller 1959, *Proconodontus* aff. *muelleri* Müller 1969 and the Lower Trempealeau zonal index²⁰, *Proconodontus posterocostatus* Miller 1980. Also present is a compound conodontophorid with an erect, laterally compressed cusp and wide basal cavity which is referable to the Tremadoc genus *Iapetognathus* Landing 1982 (Fig. 3) known from North America and Sweden. On present evidence^{20,21} *Proc. posterocostatus* is restricted to the Lower Trempealeau (Upper Cambrian) and *Iapetognathus* to the Tremadoc (Lower Ordovician). The remaining conodonts are mainly Upper Cambrian species though some range into the Ordovician. It is probable that the abundant carbonate intraclasts within the limestone block contain the Upper Cambrian conodonts; judging from its unusually abraded and pitted nature, the *Iapetognathus* may have been reworked also. Therefore the conodont fauna provides only a lower age limit of Lower Tremadoc on the limestone block. An upper age limit of Upper Ordovician is provided by the asaphids²².

Whole rock K–Ar radiometric dating²³ on slates in the Robertson Bay Group yield ages ranging between 450 and

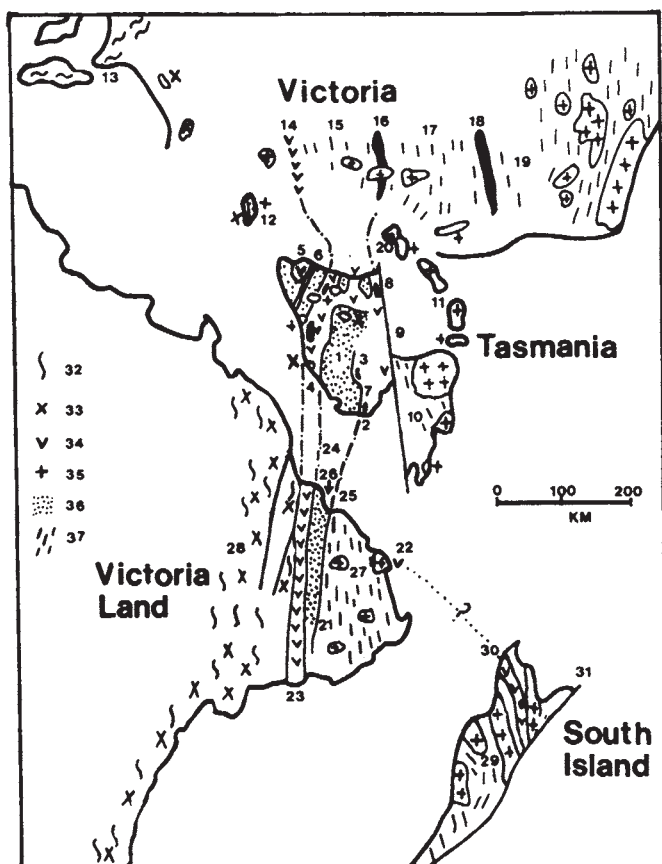


Fig. 4 Possible reconstruction of major blocks prior to Cretaceous break-up of Gondwanaland. 1, Tyennan Block; 2, South Coast Tasmania; 3, Adamsfield Trough with ultramafics; 4, Dundas Trough; 5, Smithton Basin; 6, Arthur Lineament; 7, Lake Edgar-New River Fault; 8, Beaconsfield ultramafic and Cambrian sediments and volcanics; 9, Tamar Fracture; 10, Mathinna Beds; 11, Bassian Rise granites; 12, King Island; 13, Kanmantoo Fold Belt; 14, Stavelly Greenstone Belt; 15, Ballarat Trough; 16, Heathcote Greenstone Belt; 17, Melbourne Trough; 18, Mt Wellington Greenstone Belt; 19, East Victorian Metamorphic Belt; 20, Cape Liptrap-Waratah Bay; 21, Handler Ridge; 22, Unger Island volcanics; 23, Bowers Trough; 24, Leap Year Fault; 25, Lillie Glacier Fault; 26, Millen Range schist; 27, Robertson Bay Group; 28, high grade metamorphics, migmatites of the Wilson Group and correlates; 29, Greenland Group; 30, Central Belt of Cambrian volcanics and sediments (N. W. Nelson); 31, Alpine Fault; 32, relatively high grade metamorphics of the Wilson Group in Victoria Land and the Kanmantoo Fold Belt in South Australia; 33, late Precambrian-Ordovician granitoids; 34, Cambrian volcanic and siliciclastic sequences; 35, Devonian-Lower Carboniferous granites; 36, metamorphics of the Tyennan Block and the Millen Range; 37, relatively low grade turbiditic Palaeozoic fold belts of Victoria, Tasmania and New Zealand.

510 Myr. These ages are thought²³ to be metamorphic ages related to the Cambro-Ordovician Ross Orogeny, and therefore the sequence at Handler Ridge can be no younger than 450 Myr (that is, no younger than Llandeilo²⁴).

The Robertson Bay Group, a simply-deformed sequence of Lower Ordovician and ?Cambrian turbidites intruded by Late Devonian granites, invites comparison with the Lower Ordovician-Lower Devonian turbiditic Mathinna Beds²⁵ of eastern Tasmania which are also intruded by Late Devonian granites. However, the Mathinna Beds contain neither conglomerates nor limestones, and a closer similarity appears to be with the recently described Wierah Formation on the south coast of Tasmania^{26,27}. This formation overlies disconformably a sequence of turbidites belonging to the Point Vivian Formation. Thin limestone beds from the turbiditic lower part of the Wierah

Formation contain an uppermost Cambrian conodont/phosphatocopine ostracod/brachiopod/trilobite fauna.

There is a considerable stratigraphic gap (~500 m) above the Wierah Formation but it is succeeded at Surprise Bay by a deep-water turbiditic carbonate sequence of Caradoc age (Upper Middle Ordovician)^{28,29} in contrast to the peritidal carbonates that covered the western half of Tasmania in the Middle-Upper Ordovician.

Sedimentological evidence from the Upper Cambrian and Ordovician sediments on the Tasmanian south coast indicates palaeoslopes towards the south and southeast (refs 28 and 29 and K. Bischoff, personal communication). Derivation of Australian sediments, during the Ordovician, from the Robertson Bay Group, as suggested recently³⁰, is therefore unlikely. Instead, an Upper Cambrian to Ordovician basin may have existed to the (present day) south of Tasmania, parts of which are now present in southern Tasmania, North Victoria Land, and could be represented also by the Lower Ordovician turbiditic³¹ Greenland Group of South Island, New Zealand (Fig. 4).

If the Robertson Bay Group and the Wierah Formation are aligned as in Fig. 4 then the Bowers Trough (no. 23 in Fig. 4), consisting of a Cambrian siliciclastic and volcanic sequence is aligned with the geologically similar Dundas Trough (no. 4 in Fig. 4) in western Tasmania. This alignment has been suggested several times before^{8,14,16}. The recently proposed Lillie Glacier Fault¹⁹ in North Victoria Land (no. 25 on Fig. 4) is aligned with the Lake Edgar-New River Fault (no. 7 on Fig. 4) and the newly described¹⁵ schists in the Millen Range (no. 26 on Fig. 4) are aligned with the Precambrian metamorphics of the Tyennan Block (no. 1 on Fig. 4). Eastern Tasmania, along with the Bassian Rise granites (no. 11 on Fig. 4) and the Lower Palaeozoic sequence of Waratah Bay-Digger Island³² in Victoria (no. 20 on Fig. 4) are displaced along the Tamar Fracture²⁵ (no. 9 on Fig. 4).

Future work in the Robertson Bay Group will assist delineation of the extent of the Lower Ordovician sediments and their relationships to surrounding areas.

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Biomass conversion to liquid fuels: potential of some Arizona chaparral brush and tree species

Edwin A. Davis*, James L. Kuester†
& Marvin O. Bagby‡

* Rocky Mountain Forest and Range Experimental Station, Forestry Sciences Laboratory, and † Department of Chemical Engineering, Arizona State University, Tempe, Arizona 85287, USA
‡ Agricultural Research Service, USDA, Northern Regional Research Center, Northern Agricultural Energy Center, Peoria, Illinois 61604, USA

Arizona chaparral brush and tree species can be converted into high-grade liquid hydrocarbon fuels by indirect liquefaction in which pyrolysis is followed by catalytic liquefaction. Here we have examined various Arizona chaparral species and other woody plants for oil and hydrocarbon content and as biomass feedstocks for pyrolytic conversion to high-grade liquid fuels. Although no tested species contained enough oils or hydrocarbons to be considered useful sources, gasification data show that they are all promising biomass feedstocks for pyrolytic conversion to liquid fuels. Olefin yield from biomass gasification, which is an indicator of liquid hydrocarbon yield, may be influenced by feedstock composition. If some particularly outstanding biomass feedstocks were discovered, then manipulation of the vegetation to favour those species could be a management alternative to prescribed burning or chemical control. Because most of the chaparral species tested produced pyrolysis gas of comparable olefin content, however, harvesting of chaparral without regard to species composition is indicated.

Brushlands in the southwestern United States have been viewed as a low-value, relatively unproductive resource at best^{1–5}, or an extreme fire hazard at worst^{6–9}. Attempts have been made to improve the productivity of chaparral and other brushlands using brush control treatments for the purpose of increasing streamflow duration and amount^{10,11}, augmenting forage for grazing^{10,12,13}, improving deer habitat^{14,15}, and reducing the spread of wildfires^{16,17}. Under present practices these benefits must justify expenditures for treatment costs. Of the various benefits, increased grazing capacity generates the only return on capital investment. In the process of controlling brush, either through the use of prescribed burning or chemicals, a potentially valuable energy resource is sacrificed. An alternative to this process would be to harvest the brush on a rotation basis as a renewable energy resource for conversion into liquid fuels, densified fuelwood, or energy-rich chemicals.

In the present study, plant material for testing consisted of branches, stems and leaves of mature shrubs. The samples were reduced in a series of grinding steps (chipper, hammer mill and Wiley mill) to pass through a 6-mm screen. Analytical samples were ground still further to pass through a 1-mm screen. Analytical data are reported on an oven-dry basis. The solvent extraction scheme used to characterize the biomass feedstocks in terms of oil, hydrocarbon and polyphenol fractions is one used extensively by the US Department of Agriculture to discover energy-rich plants^{18,19}.

Test species were evaluated with respect to their oil and hydrocarbon contents in comparison with those of selected

reference species (Table 1). None of the brush or tree species ranked with guayule in hydrocarbon content or with gopher plant in oil content. The polyphenol fraction is of interest as an indicator of tannin content and of reactive substances potentially valuable for making adhesives, phenolic resins, antioxidants and other products²⁰. Of the species tested, sugar sumac gave the highest polyphenol value (16.3%) which compares favourably with the reference plant, smooth sumac (20.2%), once considered a commercial source of tannin²¹. Black greasewood contained considerably more crude protein than the other species; it compares well with gopher plant, which had the highest value, and with terminal twigs of several deer-browse shrubs²² even though the greasewood sample contained much more woody material relative to terminal leafy twigs. None of the test species rated well as potential hydrocarbon- or botanochemical-producing species.

Biomass conversion options include biological processes to produce methane or alcohols, and thermochemical processes to produce heat, low- or medium-quality gas, oxygenated pyrolytic oils by direct liquefaction, or high-quality hydrocarbon liquid fuels by indirect liquefaction^{23,24}. Thermochemical conversion via indirect liquefaction offers promise for converting biomass to high-grade transportation fuel equivalent to that derived from petroleum; the product would be compatible with existing engine design and the fuel distribution system. Three indirect liquefaction routes for biomass have been reported: (1) Naval Weapons Center (NWC) system²⁵; (2) Mobil's system^{26,27}; and (3) Arizona State University's (ASU) system²³. All three systems use a synthesis gas. The NWC recommended an externally heated tubular reactor; Mobil technology did not address the gasification step; and the ASU system uses a fluidized bed with a circulating solid heat transfer system which converts unfractionated synthesis gas to a diesel-type fuel in a catalytic second stage.

A wide variety of feedstocks can be processed in the ASU system to a gas with a heating value of ~500 Btu per scf (standard cubic feet). Because biomass contains very little sulphur, the hydrogen sulphide content of the gas beyond the wet scrubber is negligible. Potential products include medium-quality gas, 1-propanol, paraffinic fuels (diesel, kerosene, jet), and high-octane gasoline. A key step is to tailor the pyrolysis gas composition with respect to carbon monoxide, hydrogen and olefins for use as a synthesis gas for the liquid fuels system.

The capability of the ASU system has been demonstrated with several feedstocks, for example almond prunings (from California orchards) and guayule bagasse (from bushes in Mexico). Composition of the pyrolysis gas is of high fuel value (about 500 Btu scf⁻¹) for all feedstocks, with minor variations in gas composition²⁸. The water-alcohol phase for both feedstocks from the liquefaction reactor typically contains ~90% water by weight, 9% 1-propanol, and less than 1% other alcohols. Gas chromatograms of the liquid hydrocarbon products from various feedstocks are very similar in the C₇–C₁₇ range and are similar to that of no. 2 diesel fuel; the major difference is the presence of C₇–C₉ compounds in the biomass liquid fuel products²⁸. The biomass product should be directly usable as a transportation fuel without distillation. Properties of the undistilled biomass liquid fuel match those of JP-4 jet fuel more closely than those of no. 2 diesel fuel²⁸.

Factor studies in the laboratory have shown that pyrolysis gas composition is an adequate predictor of the capacity of biomass feedstocks to yield liquid fuel. Indicators of a high-quality pyrolysis gas are high olefin content and a H₂/CO ratio of 1.0–1.5. Most dry biomass feedstocks without water addition yield a H₂/CO ratio of 0.5–0.9. This ratio can be increased to the 1.0–1.5 range (and higher) by adding steam to the pyrolysis reactor fluidization gas to produce hydrogen and to deplete carbon monoxide via the water gas shift reaction (CO + H₂O → H₂ + CO₂), with the reaction possibly catalysed by ash from the feedstock²⁸. The optimal pyrolysis reactor fluidization gas appears to be a combination of steam and liquefaction reactor off-gas to supply higher molecular weight paraffinic hydrocar-

Table 1 Biomass feedstock analysis and gasification results

Species	Biomass feedstocks	Common name	Feedstock analysis					Operating conditions			Pyrolysis reactor performance						
			Ash (%)	Crude protein (%)	Polyphenol (%)	Oil (%)	Hydrocarbon (%)	Heating value (Btu lb ⁻¹)	Fluidizing gas (lb h ⁻¹)	Temp. (°C)	Residence time (sec)	Gas composition (mol%)					
												H ₂	CO	CO ₂	Paraffins	Olefins	H ₂ /CO ratio
Range and woodland feedstocks																	
<i>Quercus turbinella</i>		Shrub live oak	3.1	4.6	7.4	1.1	0.1	8,524	22	760	5.2	28.0	41.3	4.5	18.0	8.1	0.67
<i>Cercocarpus betuloides</i>		Mountain mahogany	3.1	4.8	8.1	1.2	0.1	9,076	22	749	4.2	27.6	37.8	4.8	16.9	12.8	0.72
<i>Arctostaphylos pungens</i>		Pointleaf manzanita	1.7	2.0	10.4	0.9	0.3	8,444	22	749	5.2	24.5	35.5	10.6	16.3	12.8	0.68
<i>A. pringlei</i>		Pringle manzanita	2.2	2.1	11.4	0.6	0.3	8,775	22	760	4.6	25.0	40.7	6.8	16.4	11.1	0.61
<i>Garrya wrightii</i>		Wright siltassel	3.2	1.8	4.6	2.6	0.2	9,265	22	716	4.7	25.6	38.7	5.4	18.3	11.9	0.66
<i>Rhus ovata</i>		Sugar sumac	5.3	3.7	16.3	2.8	nil	—	22	649	4.1	28.9	31.9	10.6	18.2	10.4	0.90
<i>Larrea tridentata</i>		Cresosote bush	3.8	6.4	5.7	0.5	0.1	8,822	22	749	4.5	26.0	39.4	7.7	16.6	10.2	0.65
<i>Prosopis velutina</i>		Velvet mesquite	4.4	6.5	4.8	1.0	0.1	8,507	21	921	3.5	33.0	44.4	5.1	18.5	5.0	0.74
<i>Cupressus arizonica</i>		Arizona cypress	4.5	1.6	9.5	2.0	nil	7,955	22	732	4.6	26.6	38.4	7.0	17.5	10.3	0.69
<i>Juniperus osteosperma</i>		Utah juniper	4.2	3.4	6.7	1.3	0.2	8,589	22	704	4.5	28.8	39.5	6.4	17.9	7.3	0.72
<i>Pinus edulis</i>		Pinyon pine	3.3	2.6	5.0	3.0	0.3	9,060	22	771	5.3	25.8	41.8	4.4	17.6	10.0	0.61
<i>Sarcobatus vermiculatus</i>		Black greasewood	5.0	10.6	2.5	0.6	0.1	—	—	—	—	—	—	—	—	—	—
Reference feedstocks																	
<i>Pseudotsuga menziesii</i>		Douglas-fir (bark)	5.1	—	17.2	5.0	0.2	10,076	23	760	4.0	16.6	53.4	3.0	19.7	7.1	0.31
<i>Prunus amygdalus</i>		Almond (prunings)	2.8	—	7.3	0.6	0.3	8,235	22	727	5.2	23.0	44.9	6.6	16.4	9.2	0.51
<i>Parthenium argentatium</i>		Guayule (raw)	5.2	4.3	8.9	2.3	10.4	10,820	20	710	4.6	17.3	35.0	8.5	28.6	10.6	0.49
<i>P. argentatium</i>		Guayule (bagasse)	3.9	3.7	5.9	2.0	4.5	7,838	21	593	6.5	19.7	38.7	7.1	24.1	10.2	0.51
<i>P. argentatium</i>		Guayule (cork)	2.1	6.2	6.6	6.0	2.3	11,376	22	704	7.5	20.6	22.1	3.8	31.1	22.3	0.92
<i>Euphorbia lathyris</i>		Gopher plant	3.1	12.7	7.6	9.9	0.4	8,725	31	743	6.4	33.0	26.3	7.6	18.7	14.1	1.26
<i>Rhus glabra</i>		Smooth sumac	7.7	7.1	20.2	5.9	0.2	—	—	—	—	—	—	—	—	—	—

The analytical values are based on a moisture-free whole plant basis. Ash and crude protein (calculated as Kjeldahl nitrogen times 6.25) were determined for separate subsamples. The pyrolysis fluidized medium was sand, and the fluidizing gas was recycled pyrolysis gas.

bons for cracking to olefins and hydrogen. Liquid fuel yields of ~40 gallons per ton of biomass can be expected when the pyrolysis gas has an olefin content of ~10 mol%.

A variety of rangeland brush and tree species representative of chaparral, desert shrub and woodlands in Arizona were tested through the gasification step. All biomass feedstocks were tested using recycled pyrolysis gas at 21–22 lb h⁻¹ without steam addition. Although these conditions are not considered optimal for maximizing liquid fuel production, a relative assessment of the feedstocks can be made. Reference feedstocks were tested in similar conditions. The olefin content of the pyrolysis gas from 11 range and woodland species varied from 5 to 13 mol% (Table 1). Pointleaf manzanita, birchleaf mountain mahogany, Wright siltassel and Pringle manzanita yielded the greatest amounts of olefins. Velvet mesquite and Utah juniper yielded the smallest amounts of olefins and compare closely with the reference feedstocks, fir bark and almond prunings. Outstanding olefin yield was obtained from the reference feedstock guayule cork, which thus far is superior to all other biomass feedstocks tested, including raw guayule and guayule bagasse. This finding was the basis of the effort to identify high olefin-yielding biomass feedstocks and to correlate chemical composition with olefin yield. Rank correlation analysis did not indicate significant correlation between olefin yield and the other variables. A detailed chemical characterization of guayule cork may prove helpful in identifying plant constituents that correlate with high olefin yields in the pyrolysis gas. This in turn could provide a key to discovering high olefin-yielding plants.

We conclude that relatively unproductive chaparral and woodlands are a valuable renewable energy resource from which high-grade liquid fuels can be produced. Side benefits from harvesting these vegetation types would be similar to the main benefits obtained from prescribed burning: range improvement for cattle and wildlife, temporary increases in water yield, and fire hazard reduction. An important difference, however, is that harvesting the biomass for fuel production recovers the stored solar energy, whereas prescribed burning or chemical control methods waste it. Of course, many situations would occur where, because of slope steepness or terrain, harvesting would be impracticable and burning or chemical control would be the method of choice. Conversions from brush to grass in which the brush is killed by means of chemicals offer many years of increased water yield^{10,11}, but an associated side effect is an increase in the nitrate concentration of the stream water²⁹. Harvesting of resprouting brush should not result in the loss of nitrate from the watershed soil to the stream water because the nitrogen cycle would not be broken as it is when the brush is killed.

The problems associated with a programme of harvesting brush to make fuel are being addressed and prospects for their solution are encouraging^{30–34}. The design of an operational mobile biomass conversion system that is self-sustaining with respect to energy is essential for range biomass harvesting to be economically feasible. The California mobile pyrolysis unit is an example of such a unit being built to produce low-Btu gas and pyrolytic oil³⁵. However, the primary product of this system is a highly oxygenated oil that would require considerable refining to be useful as a transportation fuel. Research on harvesting rotations for maximum sustained yields and fertilization requirements to replace nutrients lost through harvesting will eventually be needed. Of immediate importance is the demonstration of the operational validity of the concept. The research reported here is just a beginning in that it demonstrates that rangeland brush and trees can be converted into high-grade liquid fuels.

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Shallow-water sclerosponges on Jamaican reefs and a criterion for recognition of hurricane deposits

Terence P. Scoffin

Department of Geology, University of Edinburgh,
Edinburgh EH9 3JW, UK

Malcolm D. Hendry

Department of Geology, University of West Indies, Mona, Kingston,
Jamaica, West Indies

We report here that excavation into the *Acropora palmata* rubble on the reef crest at 4 m water depth on Discovery Bay reefs, Jamaica, revealed the presence of numerous sclerosponges (*Ceratoporella nicholsoni*) living attached directly to the coral branches 1-2 m below the rubble surface. The coral branches excavated were deposited during a storm event (in August 1980) and consequently lack the zoned crusts showing a succession from photophilic (light-loving) to sciaphilic (shade-loving) secondary frame-builders which normally develop during slow steady incremental coral branch accumulation. Thus the nature and sequence of encrustations on coral rubble can indicate the speed of its accumulation.

Before 1980 the shallow reef front (0 to 7 m water depth) of the Discovery Bay west fore-reef consisted predominantly of erect, thick branches of living *A. palmata*^{1,2}. In August 1980

large waves generated by hurricane Allen struck these reefs^{2,3} and the bulk of the *Acropora* corals were broken and many subsequently died. In July 1983, although there had been some recovery of *Acropora* corals, there was mainly a loose pile (estimated at 0.5-2 m thick) of dead *A. palmata* rubble occupying the shallow reef front and extending leeward into ramparts exposed up to 1.5 m above sea level. We excavated a hole, at a water depth of 4 m, into the loose rubble of the reef crest to reveal the internal structure of the accumulation and to note the type and nature of encrusting organisms.

The hole excavated was 3 m in surface diameter and 2 m deep, and free of sediment less than cobble size. The dead *A. palmata* plates extracted ranged from 30 to 150 cm long, 15 to 40 cm wide and 1 to 10 cm thick. They were stacked at all angles but most lay sub-horizontally. A depth zonation of encrusting organisms was clearly evident as the hole was deepened to a depth representing a vertical stack of 10-15 *A. palmata* branches. Table 1 lists the principal calcareous encrusting organisms encountered; most were living at the time of examination. The organisms found in each 20 cm depth zone are listed in decreasing abundance and recorded for the upper and lower surfaces of the *A. palmata* plates. A gradual change from photophilic to sciaphilic organisms occurs with depth. The overlapping *A. palmata* plates shield areas beneath from direct light, although small gaps in the lattice of branches allowed patches of photophilic organisms to survive in the shafts of light penetrating down to a maximum depth of ~80 cm below the rubble surface. The crusts of calcareous red algae reached thicknesses of 8 mm at the surface, but below about 30 cm they were less than 2 mm thick. Of special interest was the occurrence of numerous sclerosponges (*Ceratoporella nicholsoni*), maximum diameter 6 mm, average diameter 3 mm, in abundances up to 50 per 100 cm² on the undersurfaces of *A. palmata* branches between 1 and 2 m below the rubble surface. Almost all the organisms listed in Table 1 (except some serpulids and cheilostome ectoprocts and the thicker crusts of algae near the surface) were growing directly on the dead *A. palmata* plates; they were not overlapping other groups of organisms to form composite crusts.

Although reef-encrusting organisms have differing rates of colonization of new substrates^{4,5}, 3 years is probably long enough for the succession to be complete⁵. Thus, the vertical sequence of encrusting organisms observed down the excavation is probably a stable community which, if undisturbed, would eventually form crusts of constant composition on the coral fragments.

During his study of the calcareous encrusting organisms of the Recent and Pleistocene reefs of Barbados, Martindale⁴ noted that *A. palmata* fragments were normally coated by a crust (up to 10 cm thick) consisting of thick photophilic algal layers near to the coral substrate, gradually giving way to thinner laminae of sciaphilic algae and foraminiferans, bryozoans and serpulids. Martindale interpreted this common composite succession as being a result of the progressive accumulation of *A. palmata* branches, causing a gradual reduction of light, and also a reduction in water circulation and a probable increase in turbidity. The absence of such a sequence of encrustation on the rubble of the Discovery Bay reef crest relates to the instantaneous deposition of the thick pile of fresh coral branches.

Studies of sclerosponge ecology on Jamaican reefs⁶⁻⁸ have shown that these calcareous sponges can tolerate conditions of exceedingly low light intensity and also low levels of nutrient supply. On Jamaican reefs, *C. nicholsoni* grows out in the open down to at least 200 m depth, and is the principal frame-builder between 70 m and 105 m depth⁸. Above 60 m depth it gradually becomes more cryptic as the twilight zone recedes further into the reef, and above 40 m depth it is almost exclusively cavernicolous. The occurrence of many *C. nicholsoni* skeletons encrusting the fragments of shallow-water corals indicates the existence at some previous time of a deep pile of fine sediment-free rubble, through which light could scarcely penetrate but water could circulate. The juxtaposition of the sclerosponges to relatively fresh shallow-water coral fragments indicates that the

Table 1 Principal calcareous encrusting organisms

Excavation depth	Substrate upper surface	Substrate under surface
0–20 cm	Calcareous red algae (up to 8 mm-thick crusts) <i>Porolithon</i> sp., <i>Lithophyllum</i> sp., <i>Neogoniolithon</i> sp.	Calcareous red algae (crusts <2 mm) <i>Mesophyllum</i> sp., <i>Neogoniolithon</i> sp. Encrusting foraminiferans <i>G. plana</i> , <i>H. rubrum</i>
20–40 cm	Platy corals (~10 cm diameter) (<i>Agaricia agaricites</i> , <i>Porites astreoides</i>), calcareous red algae (crusts <2 mm), <i>Porolithon</i> sp., <i>Lithophyllum</i> sp., <i>Neogoniolithon</i> sp., <i>Mesophyllum</i> sp. Delicate branching <i>Stylaster</i> sp. corals occur in concave downward-facing bends or joints of <i>A. palmata</i> branches	<i>Neogoniolithon</i> sp., <i>Mesophyllum</i> sp., <i>G. plana</i> , <i>H. rubrum</i> , <i>Carpentaria utricularis</i> , cheilostome ectoprocts, serpulids (? <i>Hydroides</i> sp. ? <i>Filigrana</i> sp.), thecidoid brachiopods
40–60 cm	<i>Lithophyllum</i> sp. <i>Mesophyllum</i> sp. <i>Neogoniolithon</i> sp. <i>Gypsina plana</i> <i>Homotrema rubrum</i> Cheilostome ectoprocts <i>Agaricia agaricites</i>	<i>Mesophyllum</i> sp. <i>Neogoniolithon</i> sp. <i>H. rubrum</i> Cheilostome ectoprocts <i>G. plana</i> Serpulids <i>C. utricularis</i>
60–80 cm	<i>Mesophyllum</i> sp. Serpulids <i>H. rubrum</i>	Cheilostome ectoprocts Serpulids Thecidoid brachiopods
80–100 cm	Nestling sponges ? <i>Orina calcinea</i> <i>H. rubrum</i> Cheilostome ectoprocts Serpulids <i>C. nicholsoni</i> (2 per 100 cm ²) Solitary coral (? <i>Astrangia</i> sp.) Nestling sponges ? <i>Orina calcinea</i> ?Rhizaminid foraminifera	<i>C. nicholsoni</i> (7 per 100 cm ²) Cheilostome ectoprocts Serpulids <i>C. nicholsoni</i> (20 per 100 cm ²) <i>H. rubrum</i> Thecidoid brachiopods
100–120 cm	Cheilostome ectoprocts <i>H. rubrum</i> Serpulids <i>C. nicholsoni</i> (4 per 100 cm ²) Solitary corals (? <i>Astrangia</i> sp.) ?Rhizaminid foraminifera	Cheilostome ectoprocts Serpulids <i>C. nicholsoni</i> (30 per 100 cm ²) <i>H. rubrum</i> Thecidoid brachiopods
120–140 cm	Serpulids Cheilostome ectoprocts <i>C. nicholsoni</i> (20 per 100 cm ²) Thecidoid brachiopods	<i>C. nicholsoni</i> (50 per 100 cm ²) Thecidoid brachiopods Solitary corals (? <i>Astrangia</i> sp.) <i>H. rubrum</i> Serpulids
140–160 cm	Serpulids <i>C. nicholsoni</i> (20 per 100 cm ²) Cheilostome ectoprocts	Cheilostome ectoprocts <i>C. nicholsoni</i> (50 per 100 cm ²) Cheilostome ectoprocts
160–180 cm	<i>C. nicholsoni</i> (5 per 100 cm ²) Serpulids	<i>C. nicholsoni</i> (20 per 100 cm ²) Serpulids
180–200 cm	Thin cover of sand-sized sediment Encrustations of platy corals, <i>A. agaricites</i> (dead)	<i>C. nicholsoni</i> (10 per 100 cm ²) Serpulids

pile of rubble accumulated at one depositional event, otherwise a progressive sequence from photophilic to sciaphilic encrusters, would be expected on each coral fragment.

Presently, in sheltered hollows at the surface of the rubble, 10-cm diameter platy corals (*Agaricia agaricites* and *Porites astreoides*) are attached to, and to an extent binding, the *A. palmata* fragments. Unless broken by another major storm, these platy corals, with time, should be preserved as a crudely horizontal platy encrustation on the top of the storm rubble but beneath further 'calm weather' deposits of *A. palmata*, which, no doubt, will soon cover the reef crest. The occurrence of dead encrustations of platy *A. agaricites* at the base of the excavation suggests that we had reached the top of the previous increment of rubble.

Clearly, the depositional histories of cliff or core sections of coral rubble cannot be completely unravelled by studies of the fragments' texture and disposition alone; the nature and sequence of assemblages of encrusting organisms, however, can help to elucidate the rate of supply of coral branches.

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Real and apparent movement nulled

R. L. Gregory & J. P. Harris

Brain and Perception Laboratory, Department of Anatomy, Medical School, University of Bristol, Bristol BS8 1TD, UK

It is a controversial issue whether 'real' movement, seen from the successive stimulation of retinal receptors by a moving image, and 'apparent' (phi) movement, which is produced by stimuli separated in space and time, are transmitted by the same or different neural channels. With this question in mind, we have devised ways to oppose real against opposite-direction apparent movement, to see whether they cancel. Cancelling, especially in a variety of conditions, would be expected for a single but not for separate neural movement channels. As we report here, we find that opposed-direction real and apparent movements will cancel, allowing null measurements to be made.

The simplest method is to rotate a sector disk at constant speed, in, say, a clockwise direction, and illuminate it with two sources—flashes of light at a constant rate, and a continuous light. An electronic stroboscope provides short (1 μ s) flashes which are set at a repetition rate to make the sector disk rotate apparently backwards (anticlockwise). The continuous light signals the disk's true (clockwise) rotation as for normal viewing. As the stroboscope flashes are short, the retinal images of the

sectors are essentially stationary for each flash, and so only apparent movement is signalled (anticlockwise) while the continuous light signals clockwise real movement. The question is: is there an intensity ratio of the intermittent and continuous lights at which the opposed real and apparent movements cancel? We find that there is a critical balance of intensities at which systematic movement ceases. At this cancelling null point, all that remains is rapid flicker, or a random jiggle. This is phenomenally similar to what happens when two oppositely moving gratings are viewed superimposed, to cancel two real movements. The finding that opposed real and apparent movements can cancel suggests that they are carried by the same neural channel.

At a lower relative intensity of the continuous light, the apparent movement from the stroboscope dominates and the disk rotates anticlockwise; at higher intensities, it rotates clockwise by real movement. The mean luminance, which is not critical, was in the lower photopic range.

As the disk can have any number of equally spaced sectors, and as the apparent movement occurs at several flash rates, there are many independently controllable parameters, which is exceedingly useful. The real edge velocity of the sectors, given by the rotational velocity of the disk, may be varied independently of the apparent movement, as for any rotational velocity this is given by the number of sectors and the flash rate which are independently variable. For some uses, a band of bars is better than a sector disk, which may have its centre blanked off to restrict the range of velocities along the radii of the sectors. The disks were rotated between 10 and 100 r.p.m., as selected with a stepped ratio gear box driven by a synchronous motor.

It is interesting that with large jumps of the flashed sectors, although apparent movement is maintained cancelling does not occur. The appearance is of two oppositely rotating disks passing through each other, both movements being seen, rather than cancelling. Thus, it seems that only 'short-range' movement¹ will cancel for nulling, and that 'long-range' movement², which does not cancel, is signalled by different neural channels. This occurs, for example, in the following conditions: six equally spaced white and six black sectors on an 18-cm disk, rotating at 50 r.p.m., viewed from 2 m. Illumination is: flashes of $1 \mu\text{s}$ at 8 flashes per s, with a peak luminance of about 10 Cd m^{-2} . This flash rate gives the highest attainable apparent velocity in this situation. The displacements of the signalled sector positions exceed the short-range limit for most of their radii. The added constant light is adjusted for of 2 Cd m^{-2} . These rather critical conditions give simultaneous counter-rotations when the apparent and real movements are balanced instead of nulling. When this is viewed with a fourfold reduction of size at the eye, to bring the flash image jumps within short-range movement, it is seen as nulled rather than as opposed movements.

In the more generally holding conditions for nulling, when colour filters are added, to make, say, the stroboscope flashes blue and the continuous light red, nulling still occurs in spite of colour contrast between the opposed real and apparent movements. Following prolonged fixation of this double-colour nulled movement, no significant after-effect appears, either for white light or when the stationary test pattern is viewed with blue or with red light. These observations provide further evidence that the real and apparent movements are being transmitted by the same neural channel, and that the movement channel is independent of the colour systems. This is confirmed by the observation that, following several minutes' fixation of the rotating disk set up with nulled movement, there is no significant movement after-effect. As would be expected, after-effects are seen in the opposite directions to the adapting real or apparent movements following stimulation either side of the null point.

If the nulled movement is viewed through a defocusing lens to blur the edges of the moving sectors, the null point remains essentially unchanged. However, when viewed through a neutral density filter (or a pin hole), placed before the eyes so that both illuminations are reduced equally, the null point changes. The real movement from the continuous source is now less effective

than the apparent movement from the stroboscope—as now it rotates anticlockwise. This occurs dramatically with a filter of less than half a log unit, and the shift of the null point is greater with higher filter factors. The null can be restored by resetting the luminance ratio, so this effect can readily be measured. It is evidently associated with the eye's adaptation level, and so may indicate its integration time, which increases with dark adaptation; for when a bright light is shone into the eyes, producing light adaptation, the null point shifts in the opposite direction to the shift with the dark filter. This effect is greater for low illuminations of the sector disk, while the dark filter is more effective at higher illuminations, which is to be expected for an adaptation effect. The impairment of real movement may be explained in these terms, for increased integration time must impair temporal resolution of the receptors, which will affect the sequential signalling of real movement as the image runs continuously along the receptors, but can hardly affect the signalling of flashes separated beyond the eye's longest integration time, of a few milliseconds.

Nulling can be achieved without a continuous light—by lengthening the stroboscope flashes. This is done with an electromechanical shutter driven by variable-frequency and duration pulses, and arranged to interrupt a projector illuminating the disk. The real and apparent movements can now be cancelled and nulled with no continuous light, by setting the flash duration so that the real movement which is signalled during each flash equals the opposed apparent movement, given by the repetition rate. Nulling is obtained in this way by adjusting the flash duration around 5–10 ms. The dark filter changes this null point, less than for the short flash plus continuous light. In both cases increase in the eye's integration time with dark adaption impairs continuous movement without impairing the apparent movement, at least for these low flash rates of under 10 Hz.

Repeated texture patterns, in place of the sectors, should allow pattern-signalled movement to be compared with edge signalled movement. It has been shown that apparent movement is lost with isoluminant colour contrast for alternating texture patterns, though not for clearly separate features or objects³. The movement nulling technique will be used with isoluminant colour contrast displays, both with repeated texture patterns and with clearly separated sectors or dots, to explore these effects of luminance and colour contrast for textures and individual features, both with the short-range movement, which allows cancelling and nulling, and with long-range movement which, as it does not cancel, seems to work by different neural mechanisms from those of short-range apparent and real movement.

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Induction of the ipsilateral retinothalamic projection in *Xenopus laevis* by thyroxine

Sally G. Hoskins* & Paul Grobstein

Departments of Biology and of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, Illinois 60637, USA

Hormones are important in the development of behaviour and there is now abundant evidence that they also affect the morphological development of the nervous system^{1–6}. In principle, hormones could act by inducing new patterns of connectivity between widely separated structures in the nervous system or by influencing local connectivity. Most available work documents effects of the latter sort. We present here evidence for

* Present address and address for reprint requests: Department of Biological Sciences, Columbia University, 901 Fairchild Center, New York, New York 10027, USA.

the former. Our results indicate that thyroxine, the hormone which causes metamorphosis in the frog *Xenopus laevis*, can induce precociously in a pre-metamorphic tadpole the ipsilateral retinothalamic projection, a retinofugal pathway which normally develops during metamorphosis. Our evidence suggests that the hormone's presence in one eye alone is sufficient to cause axons of some ganglion cells in that eye to grow to targets in the ipsilateral thalamus and to form terminal fields of appropriate morphology. Since the axons of the induced pathway project ipsilaterally, unlike axons in the normal pre-metamorphic tadpole, virtually all of which project contralaterally, our results are also relevant to questions concerning the control of axonal trajectory in the optic chiasm.

The ipsilateral retinothalamic projection in *X. laevis* first appears during metamorphosis⁷⁻⁹. In adult frogs, the projection arises primarily from a temporo-ventral subregion of the retina^{8,10} and terminates in several nuclei of the rostral and caudal thalamus¹¹. We have studied the development of the projection using anterograde transport of horseradish peroxidase (HRP). A complete description of this material is in preparation. Our observations, like those of investigators using ³H-proline⁸ and degeneration⁷ techniques, show the ipsilateral retinothalamic projection to be a late-developing pathway. Reaction product was either absent or extremely sparse in ipsilateral thalamic terminal zones of tadpoles at stage 54¹², approximately 3.5 weeks old (Fig. 1b). In frogs analysed 1 week after the completion of metamorphosis, about 7 weeks old, there was substantial labelling in the ipsilateral terminal fields (Fig. 1a).

To determine whether the development of the ipsilateral retinothalamic projection, like other metamorphic changes, depends on thyroxine, we reared tadpoles in 10% Holtfreter's solution and 0.01% propylthiouracil (PTU), beginning at stages 48–50. PTU prevents endogenous production of thyroxine in the thyroid gland by blocking the iodination of tyrosine¹³. PTU-reared tadpoles cease morphological development at stage 54,

but remain healthy and continue to increase in size for extended periods¹⁴. The eyes of PTU-reared animals, like those of stage 54 tadpoles, continue to grow as new cells are added symmetrically to the dorsal and ventral peripheries (Fig. 2). Despite the continuing retinal growth, the ipsilateral projections of PTU-reared stage 54 tadpoles (Fig. 1c) remain like those of normal tadpoles at stage 54. When released from PTU, the giant tadpoles complete metamorphosis normally and develop typical ipsilateral retinothalamic projections.

To confirm the thyroxine requirement for development of an ipsilateral projection, we pressure-injected small amounts (3–17 ng) of L-thyroxine in 50 nl Wesson oil into one eye of PTU-reared tadpoles using a calibrated glass micropipette, and continued to house the animals in PTU. Other PTU-reared tadpoles were injected intraocularly with oil alone. Between 3 and 12 weeks after the injection, the optic projection from either the treated or untreated eye was examined by application of HRP to the optic nerve. Some of the tadpoles, particularly those which had received higher doses of thyroxine, advanced slightly in developmental stage during the experimental period, progressing to stages 54–57 (22 cases); other animals, which had received lower doses of thyroxine, or oil alone, remained by all external criteria at the developmental stage characteristic of PTU-reared animals (7 cases).

In 14 of the animals which had advanced slightly in stage, the ipsilateral projections made by the hormone-treated eye were assessed. In 10 of these cases, the optic projection to the ipsilateral thalamus (evaluated by intensity and distribution of label in the thalamic terminal zones as described below) was clearly better developed than that of normal tadpoles at comparable stages (stages 55–57). In several instances, these ipsilateral

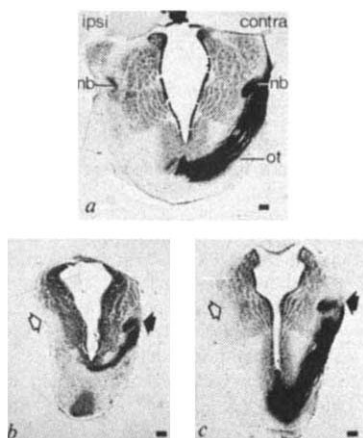


Fig. 1 The retinothalamic projection in *X. laevis* 1 week after the conclusion of metamorphosis (a), at tadpole stage 54 (b) and in a stage 54 tadpole which had been reared in PTU for 25 weeks (c). We applied crystals of horseradish peroxidase (HRP) to the central stumps of severed optic nerves. 24–48 h later, the animals were killed and the brains prepared for sectioning. Frozen sections (40 μ m) were cut on a sliding microtome, and processed using 0.5% cobalt chloride and 1% diaminobenzidine (DAB) solutions for histochemical demonstration of the HRP reaction product²². Photomicrographs are of transverse sections through the rostral thalamus at the level of the nucleus of Bellonci, the largest thalamic terminal field, in animals in which one optic nerve was exposed to HRP. In all three cases, reaction product is present in the optic tract (ot) and in the nucleus of Bellonci (nb; solid arrows) on the contralateral (contra) side of the brain. In the post-metamorphic case, but not in the two tadpoles, reaction product is also evident in the ipsilateral (ipsi) nucleus of Bellonci (nb; open arrows). Scale bars: 100 μ m.

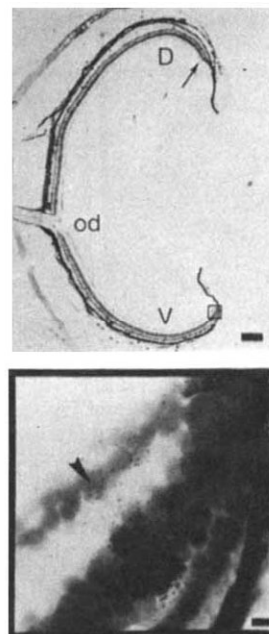


Fig. 2 Retinal growth during PTU-rearing. This PTU-reared tadpole, whose ipsilateral retinothalamic projection is illustrated in Fig. 1c, received an intra-abdominal injection of ³H-thymidine (5 μ l, specific activity 81.6 Ci mmol⁻¹, NEN), and was subsequently returned to PTU. Eleven weeks later, HRP was applied to one optic nerve of the animal, and its retinal projection was analysed. At this time, the eyes were fixed separately, sectioned at 10 μ m and processed for autoradiography. The upper part of the figure is a parasagittal section through one retina, taken at the level of the optic disk (od). Dorsal (D) and ventral (V) are indicated. The lower part of the figure is a higher magnification view of the boxed region of the ventral periphery, showing ³H-thymidine-labelled retinal ganglion cells (arrowhead) peripherally and unlabelled cells centrally. The location of a similar border in the dorsal retina is indicated by the arrow in the upper part of the figure. Scale bars: 100 μ m (upper), 10 μ m (lower).

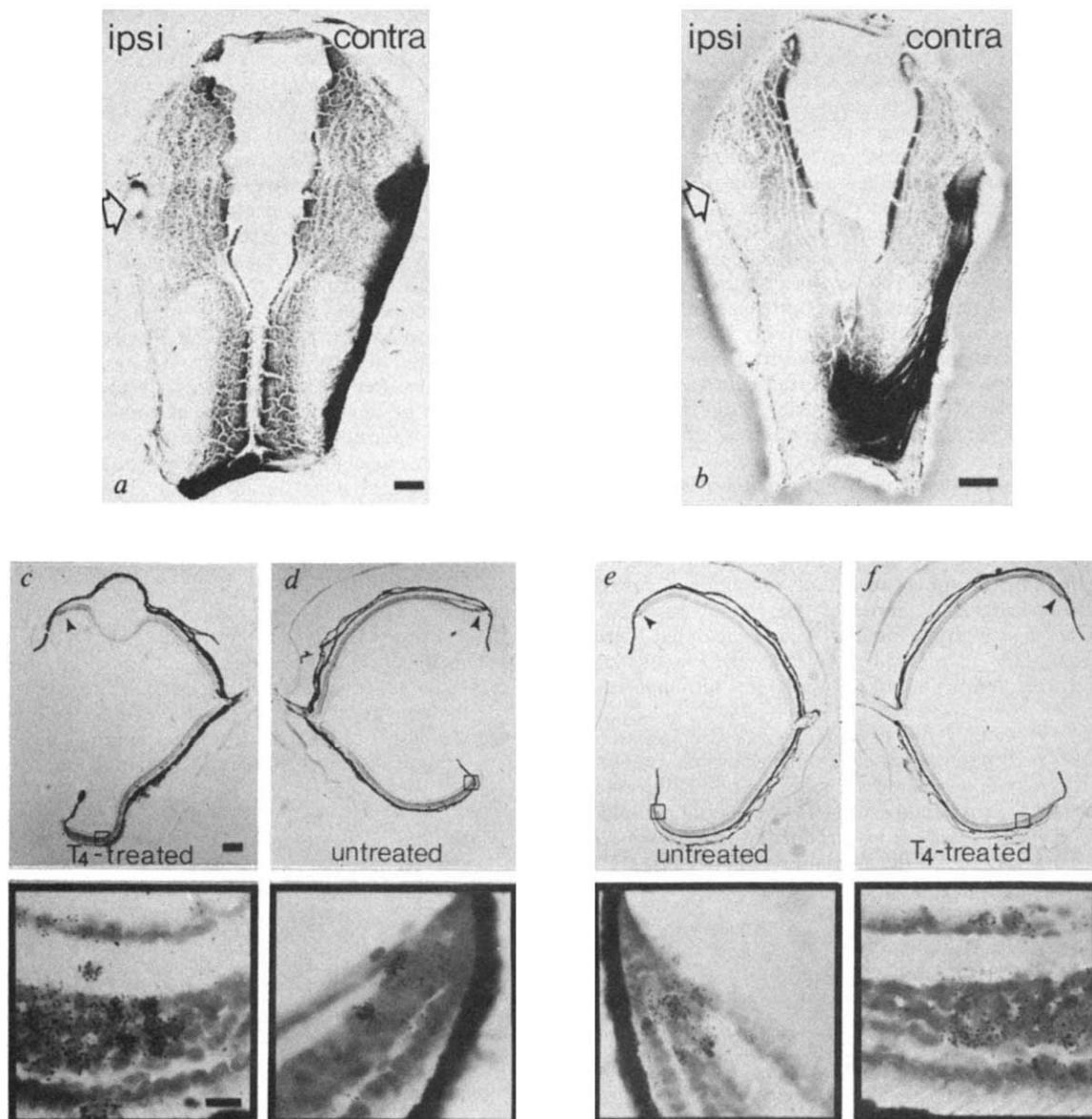


Fig. 3 Effects of injecting thyroxine into one eye of PTU-reared tadpoles. Each animal received an intraocular injection of thyroxine within 24 h of a previous intra-abdominal injection of ^3H -thymidine, like that described in the legend to Fig. 2. Photomicrographs are of brain and retinal sections oriented like those of Figs 1 and 2. The sections on the left are from a stage 54 PTU-reared tadpole in which the optic projection from the thyroxine-treated (T_4 -treated) left eye was examined following 3 weeks of further rearing in PTU. Sections on the right are from a similar case in which the right eye was thyroxine-treated and the projection from the untreated left eye was examined following 3.5 weeks of further rearing in PTU. The thyroxine-treated left eye developed an ipsilateral projection (open arrow in *a*), while the untreated left eye did not (open arrow in *b*). In both cases, the thyroxine-treated eye added more cells ventrally than dorsally (*c*, *f*), while the untreated eye grew symmetrically (*d*, *e*). As in Fig. 2, arrows and boxes in the low-power retinal sections indicate the locations of ^3H -thymidine labelling borders as well as the regions which are shown in the higher magnification views. Scale bars: *a*–*f*, 100 μm ; insets, 10 μm .

projections resembled those of postmetamorphic frogs. Four of the tadpoles in this group had ipsilateral projections which were not enhanced, but instead had developed to the extent typical of the animals' morphological stages.

Optic nerve projections from the other, untreated eye were assessed in the remaining eight thyroxine-treated tadpoles which had progressed to stages 55–57. In seven such cases, the ipsilateral projections from the other, untreated eye were those typical of normal untreated tadpoles at stages 55–57. A single stage 56 animal of this group had an enhanced ipsilateral projection. The three tadpoles injected with oil alone showed no change in developmental stage during the experimental period, and the ipsilateral projections from the oil-injected eyes were those characteristic of stage 54 tadpoles. Thus, injections of thyroxine and oil, but not of oil alone, can cause the development of advanced retinal projections to the ipsilateral thalamus. Eyes treated directly with thyroxine were much more likely to pro-

duce enhanced projections (10 of 14 cases) than were the untreated eyes of similar tadpoles (1 of 8 cases). These results clearly indicate that exogenous thyroxine can cause development of an ipsilateral projection in tadpoles in which the endogenous production of thyroxine has been prevented⁹. They also suggest that thyroxine acts directly on the eye, since the development of the ipsilateral projection was more advanced in thyroxine-treated than in non-treated eyes, and raise the possibility that a local action of thyroxine may be sufficient to cause development of the projection.

The thyroxine-treated animals which remained at morphological stage 54 throughout the experimental period also produced enhanced projections to the thalamus ipsilateral to the thyroxine-treated eye, providing more direct evidence that local hormone action may be sufficient to induce the projection. We analysed the projection from the thyroxine-treated eye in each of three tadpoles which remained at stage 54 for 3–4 weeks

following injection of 3.75 ng of thyroxine into one eye. Figure 3a is a transverse section through the brain of one of these animals, taken at the level of the nucleus of Bellonci (NB), the largest and most heavily innervated terminal field in the ipsilateral thalamus of adult frogs. The distribution of reaction product within this nucleus in the thyroxine-treated case illustrated is similar to that found in the terminal fields of young post-metamorphic animals (Fig. 1a), being most dense dorsally and medially with a less densely labelled core. Such a differential distribution of reaction product is not seen in ipsilateral thalamic terminal zones of normal developing tadpoles until stage 60, at which time the overall density of reaction product is still quite low⁹. Similar dense, differentially distributed deposits of reaction product were seen in the ipsilateral NBs of the other two tadpoles in this group. Reaction product was also found in the other usual ipsilateral terminal zones, including the uncinata field, which is not normally innervated until stage 64 (ref. 9).

In a fourth, similarly treated animal which remained at stage 54 following intraocular injection of thyroxine, we analysed the projection from the untreated eye. The projection was typical of that seen in normal tadpoles at stage 54 (Fig. 3b). Label was absent in all ipsilateral terminal zones except NB where there was extremely sparse labelling similar to that sometimes seen in normal tadpoles at stage 54 as well as in PTU-reared animals, both untreated and oil-injected.

Beginning at stage 55, the eyes of normally developing *X. laevis* tadpoles undergo a shift in the pattern of cell proliferation, resulting in greatly increased addition of cells to the ventral as compared with the dorsal retinal periphery. This shift can be elicited precociously if thyroxine is injected into the eye of younger tadpoles¹⁵. The presence or absence of an altered pattern of retinal histogenesis thus provided an additional means of testing whether the action of thyroxine was localized.

The retinas of the thyroxine-treated eyes underwent the expected shift in pattern of histogenesis during the 3.5-week period of the experiment, while those of the untreated eyes did not (Fig. 3c-f). Dense concentrations of silver grains mark the cells which underwent their final mitotic division in the presence of the ³H-thymidine label and hence formed the border of the retina at the beginning of the experimental period. In the ventral retina of thyroxine-treated eyes (Fig 3c, f, and insets), this grain border was displaced centrally as new cells were added during the experimental period. In the dorsal retina, few new cells were added, and the grains remained near the periphery. In the untreated eyes (Fig 3d, e) of the same tadpoles, in contrast, both dorsal and ventral grain borders remained symmetrically and peripherally located, indicating that the histogenetic pattern of these untreated eyes did not change during the experimental period. The distribution of silver grains in the retinas of such untreated eyes was like that seen in both eyes of PTU-reared, untreated tadpoles.

The production of ipsilateral terminal fields like those of post-metamorphic frogs in the brains of pre-metamorphic tadpoles is a striking example of the hormonal induction of a neuronal pathway. That hormonal induction of the ipsilateral projection was not observed in an earlier, unpublished study (cited in ref. 8) may have been due to the use of triiodothyronine pellets, rather than thyroxine injections. Our evidence that an ipsilateral projection from one eye can be induced in the absence of many if not all of the metamorphic changes normally occurring when the projection develops, also has interesting implications for understanding what controls the patterns of projection made by axons at the optic chiasm.

The induction of an ipsilateral projection from one eye in the absence of a similar projection from the other clearly indicates that interactions between corresponding fibres from the two eyes¹⁶ are not essential for the development of an ipsilateral projection (see also refs 8, 10). The finding also makes it unlikely that the appearance of an ipsilateral projection can be accounted for simply in terms of a critical change at the chiasm since if this were the case enhanced projections should have been seen from both eyes of hormone-treated tadpoles. In general, our

findings suggest that the primary change responsible for the development of the ipsilateral projection is a change in the cells of the eye itself. By three criteria (external morphological stage, patterns of retinal histogenesis, and projections of the untreated eye) the action of thyroxine in the animals which remained at stage 54 was restricted to the treated eye.

Both normally and in the experimental situation described here, the development of an ipsilateral projection is correlated with an asymmetric pattern of proliferation of ganglion cells, during which growth occurs primarily in ventral retina¹⁷⁻¹⁹. Retrograde transport and birthdating studies in normal *X. laevis* indicate that the vast majority of the ipsilaterally-projecting retinal ganglion cells of post-metamorphic frogs lie in retinal regions produced late in development, after the retina has begun to proliferate asymmetrically²⁰. The region of the retina which will exhibit enhanced growth is, however, apparently determined much earlier, during embryonic stages. If eye rudiments are rotated or grafted at stages well before stage 54, both the ipsilateral projection⁸ and the ventral growth burst²¹ are later found to originate from ganglion cells descended from the region previously situated temporoventrally. The ipsilateral projection of normal frogs, then, is formed during metamorphosis by neurones whose eventual fate was determined during embryogenesis. This suggests that the role of thyroxine in the development of the ipsilateral retinothalamic projection is two-fold: to produce a new population of ganglion cells, and to permit the expression of a previous determination, one which causes the axons of some of these cells to project ipsilaterally at the optic chiasm. Whether the two events are causally related is a question warranting further investigation.

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Evolutionary relationships of ratites and carinates: evidence from ontogeny of the tarsus

C. McGowan

Department of Vertebrate Palaeontology, Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario, Canada M5S 2C6

de Beer¹ proposed that ostriches and their allies, the ratites, are degenerate birds that evolved, neotenuously, from carinates (flying birds). Others²⁻⁵ have argued for their primitiveness, but the view that ratites are derived within birds has prevailed⁶⁻⁸. The problem is soluble only when avian phylogeny

is understood, and Ostrom⁹ makes a strong case for the evolution of birds from theropod dinosaurs. Included in his evidence was the ascending process of the astragalus, a feature first shown by Huxley² to be unique to theropods and birds (Fig. 1). Ostrom's critics¹⁰, however, argue that the avian process is not associated with the astragalus, being instead an independent ossification, the pretibial bone¹¹. The present ontogenetic study reveals both processes in birds: the ascending process exemplified by ratites and the pretibial bone by carinates. This implies that, since carinates possess the unique pretibial bone, they could not have given rise to the ratites, which are primitive for this feature.

Three series of embryos were obtained (at daily intervals), for the common quail (*Coturnix coturnix*) and for the chicken (*Gallus domesticus*), and these were double stained with Alizarin red and Alcian blue¹². In addition, developing chickens, emus (*Dromaius novaehollandiae*) and ostriches (*Struthio camelus*) were monitored radiographically. Data were also obtained from X rays, embryos and immature skeletons for the ostrich, emu, kiwi (*Apteryx australis*), cassowary (*Casuarius casuarius*), red-winged tinamou (*Rhynchotis refescens*), brush-land tinamou (*Nothoprocta cinerascens*) and various carinates,

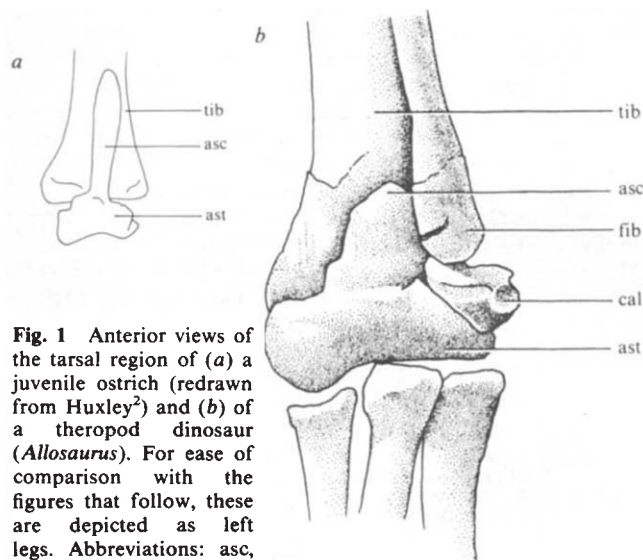


Fig. 1 Anterior views of the tarsal region of (a) a juvenile ostrich (redrawn from Huxley²) and (b) of a theropod dinosaur (*Allosaurus*). For ease of comparison with the figures that follow, these are depicted as left legs. Abbreviations: asc, ascending process; ast, astragalus; cal, calcaneum; fib, fibula; tib, tibia. Note that although the ascending process arises from the astragalus, it lies predominantly on the lateral aspect of the tibia.

specified below. Ontogeny in carinates will be considered first, followed by that of ratites.

Before ossification of the tarsus, which began on day 12 in one of the quails, and day 15 in the chickens, the distal end of the tibia is entirely cartilaginous. There are prominent lateral (calcaneal) and medial (astragalar) condyles, joined proximally, with a cartilaginous spur projecting proximally from the former (Fig. 2a; s). This spur, which is the precursor of the pretibial bone, begins ossifying from its tip (Fig. 2b-d; pre). Several days later the astragalus and calcaneum begin to ossify. These ossifications appear as small nodules embedded in their respective condyles (Fig. 2e; ast, cal). The astragalus is initially about twice as large as the calcaneum in both species. The pretibial bone eventually fuses with the (ossified) calcaneum; in the chicken this occurs some 40 days after hatching (Fig. 2f).

Similar relationships between the pretibial bone and calcaneum have been seen in stained embryos of the ring-billed gull (*Larus delawarensis*) and in radiographs of the Adelie penguin (*Pygoscelis adeliae*), common shag (*Phalacrocorax aristotelis*), common starling (*Sturnus vulgaris*), Canada goose (*Branta canadensis*), common eider (*Somateria mollissima*), mallard (*Anas platyrhynchos*), red-headed duck (*Aythya americana*), snow goose (*Chen caerulescens*) and the Mikado pheasant (*Syrnaticus mikado*). Caution is required in drawing conclusions from radiographs alone, but it seems that tarsal development in carinates is uniform.

Huxley² was sure that the ascending process of the ostrich was part of the astragalus, but this can be established only if the fate of the calcaneum is known. The youngest ostrich examined, a near-hatching embryo, has a prominent and well-ossified ascending process, expanded distally into a disk of bone occupying about half the width of the distal end of the tibia (Fig. 3a; asc, dsc). The disk is separate from, but in contact with, the medial condyle, the latter being largely ossified. The lateral condyle, with which the medial condyle is continuous, is still almost entirely cartilaginous, and is drawn up into a process which appears to be homologous with the precursor of the carinate pretibial bone (Fig. 3a; s). However, in marked contrast to carinate ontogeny this process does not ossify later. At this stage the ascending process cannot be said to be part of either astragalus or calcaneum. Radiographs of two 5-week-old juveniles show that the ascending process becomes contiguous with the astragalus, the identity of the astragalus being confirmed by the appearance of a small calcaneum lying lateral to it (Fig. 3b; cal). By the 11th week the calcaneum has fused with the astragalus (Fig. 3c). Three emu chicks, X-rayed weekly from 4 to 12 weeks after hatching, each showed a putative astragalus,

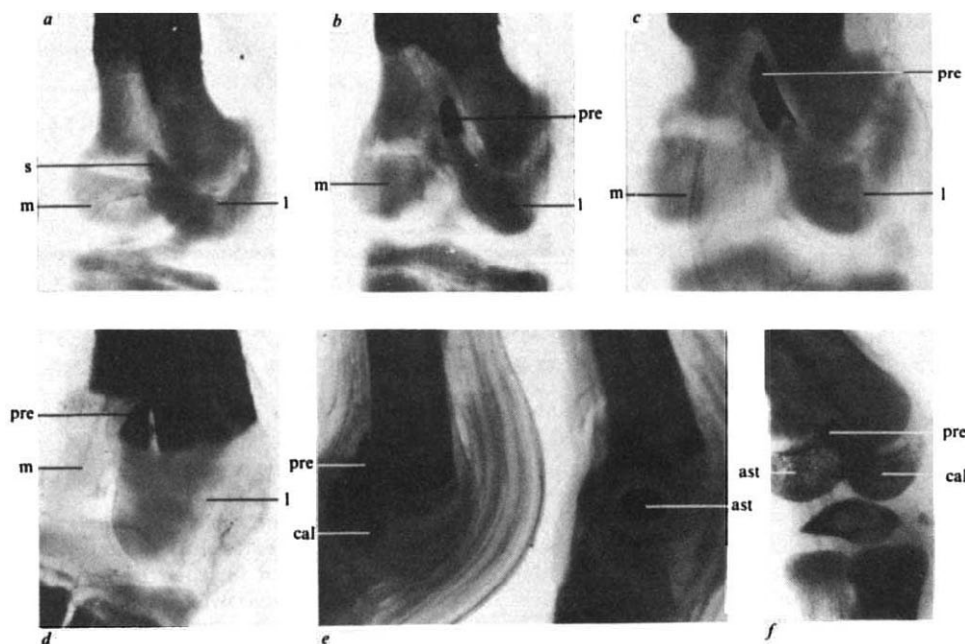


Fig. 2 Development of the carinate tarsus. Left leg depicted, unless otherwise stated. a, b, 12-day-old quail embryos, anterolateral views; c, 14-day-old quail embryo, anterolateral view; d, 19-day-old chicken, anterolateral view; e, 16-day-old quail, lateral view of left leg and medial view of right leg; f, sub-adult chicken, 40 days after hatching, anterolateral view. Abbreviations as in Fig. 1 with additions: l, lateral condyle; m, medial condyle; pre, pretibial bones; s, cartilaginous spur.

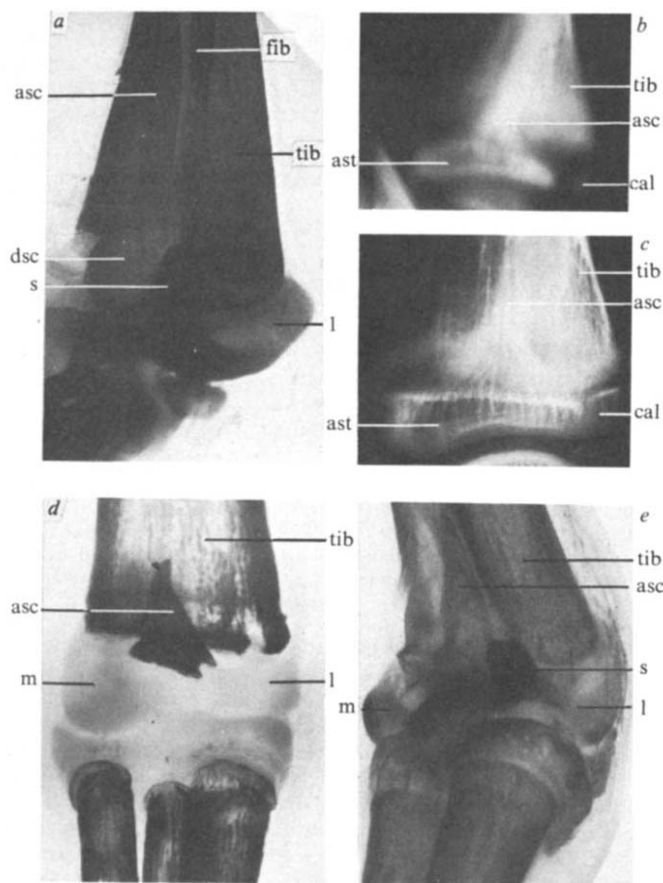


Fig. 3 Development of the ratite and tinamou tarsus. Left legs depicted. *a-c*, Ostrich; *d, e*, red-winged tinamou. *a*, Ostrich embryo shortly before hatching, anterolateral view; *b, c*, X rays of 5-week-old and 11-week-old juvenile ostriches, anterior views; *d*, late tinamou embryo, shortly before hatching, anterior view; *e*, hatching tinamou, approximately 1 week old, anterolateral view. Abbreviations as in Fig. 2, with addition: dsc, distal disk of ascending process.

with an ascending process, but no evidence of an ossified calcaneum. However, in one of four stained, near-hatching embryos, there is a small ossified calcaneum, confirming the identity of the astragalus. Radiographs of six embryonic and five subadult kiwis show a prominent ascending process but, as in the emu X rays, no evidence of an ossified calcaneum. Parker¹³, however, showed that the diminutive calcaneum had become incorporated into the astragalus, with its associated ascending process, prior to ossification. Evidence for the cassowary and rhea is presently incomplete, but radiographs show a putative astragalus with a prominent ascending process, as in the other ratites. Holmgren's¹⁴ avian studies focusing on the earlier (pre-cartilaginous) stages of development, are of limited interest here; however, he drew the erroneous conclusion that ankle development in the ostrich scarcely differed from that in the chicken, gull and duck.

Tinamous possess a mosaic of ratite and carinate features and it is therefore important to consider their tarsus. Examination of late embryos of the red-winged tinamou reveals a prominent bony ascending process lying midway between the (conjoined) lateral and medial condyles (Fig. 3*d*; asc). There are no other ossifications at this stage. In a week-old hatchling a prominent spur can be seen arising from the lateral condyle, similar to that seen in the ostrich (Fig. 3*e*; s). There is no evidence of a calcaneal ossification, but there is a small astragalus. The ascending process of the red-winged tinamou is, therefore, not an ossification of the spur from the lateral condyle, and so is not homologous with the pretibial bone of carinates but corresponds instead to the ratite condition.

There are therefore two different tarsal conditions in birds. Carinates have a pretibial bone, an ossification of a cartilaginous spur which fuses with the calcaneum. This is a synapomorphy for carinates, implying that they did not give rise to the ratites, contrary to de Beer's¹ hypothesis. Ratites (including tinamous) have an ascending process, an ossification which fuses with the astragalus, as in theropods¹⁵. The ratite tarsus is, therefore, in the primitive condition. Indeed, most of the features that distinguish ratites from carinates are now considered to be primitive rather than derived^{3-5,16}. They include the palaeognathous palate^{17,18}, a feature that has been used as evidence for neoteny¹⁹, albeit on slender evidence⁶. Ratites should therefore be viewed as primitive rather than as derived birds. This does not imply that ratites descended directly from a stage in bird ancestry before the power of flight had been acquired, only that their ancestral stock was more primitive than carinates, a point made elsewhere^{16,20}.

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Molecular genetic evidence for early evolutionary origin of budding peptidoglycan-less eubacteria

Erko Stackebrandt*, Wolfgang Ludwig*, Wolfgang Schubert†, Friedrich Klink†, Heinz Schlesner§, Telse Roggentin§ & Peter Hirsch§

* Lehrstuhl für Mikrobiologie, Technische Universität München, Arcisstrasse 16, D-8000 München 2, FRG

† Lehrstuhl für Organische Chemie I, Technische Universität München, Lichtenbergstrasse 4, D-8046 Garching, FRG

‡ Biochemisches Institut, Medizinische Fakultät der Universität Kiel, Olshausenstrasse 40-60, D-2300 Kiel, FRG

§ Institut für Allgemeine Mikrobiologie der Universität Kiel, Olshausenstrasse 40-60, D-2300 Kiel, FRG

Recent studies on the cell wall composition of budding, non-prosthecate bacteria have shown that representatives of the genera *Planctomyces* and *Pasteuria* (*sensu* Staley, 1973) lack the peptidoglycan moiety¹, which is the characteristic feature of eubacterial cell walls^{2,3}, but possess an as yet unidentified protein sheet instead; in this respect *Planctomyces* and *Pasteuria* strains resemble certain archaebacteria more than eubacteria^{3,4}. To determine their phylogenetic positions, the 16S ribosomal RNAs of three budding, peptidoglycan-less strains with and without stalks were subjected to partial

sequence analyses. As we report here, the comparison of data with those obtained from about 320 eubacterial and 40 archaeobacterial strains revealed that the strains investigated are not members of the archaeobacterial kingdom but represent an ancient line of descent of the eubacterial kingdom. Furthermore the diphtheria toxin reaction⁵ of *Planctomyces* and *Pasteuria* shows that they behave like eubacteria.

The three strains of budding, non-prosthecate bacteria investigated share the main characteristics of members of the *Planctomyces*-*Blastocaulis* group of bacteria⁶. They were isolated from brackish surface water of the Kiel Fjord, Baltic Sea¹. Strain IFAM 1317 (IFAM = Institut für Allgemeine Mikrobiologie, University of Kiel) and strain IFAM 1310 resemble *Planctomyces maris*⁷ and *Pasteuria ramosa* (*sensu* Staley, 1973), respectively, while strain IFAM 1319 shows no distinct similarity to any of the described species of this group. Cultivation of these organisms followed described methods¹. Isolation of 16S rRNA⁸⁻¹⁰ and sequence analysis¹⁰⁻¹³ were done as described. Similarity coefficients, S_{AB} values¹⁴, were calculated using a CDC computer.

Table 1 shows the similarity coefficients, calculated from the 16S rRNA oligonucleotide catalogues of the three strains investigated (not shown) and more than 350 bacterial reference strains. The S_{AB} values show strains IFAM 1310 and IFAM 1319 to be rather closely related (0.70), while they show less rRNA sequence homology with the *Planctomyces maris*-like strain IFAM 1317 (0.30 and 0.32, respectively). The degree of relatedness found between these three organisms and representatives of the various lines of descent of the eubacterial kingdom indicates that they are significantly more remote (with S_{AB} values ranging between 0.06 and 0.21, with an average value of 0.15), while almost no relationship can be detected to members of the archaeobacterial kingdom (0.04–0.12, average 0.10). The range of the latter S_{AB} values is of the same order of magnitude as that found between representatives of the eubacterial and archaeobacterial kingdoms (0.06–0.14)¹⁵. The lack of relationship between archaeobacteria and budding, non-prosthecate and peptidoglycan-less bacteria agrees with results of the diphtheria-toxin reaction, recently used to distinguish archaeobacteria and eubacteria^{5,16} (Table 2). Cell supernatants of strains IFAM 1310 and IFAM 1317 do not contain proteins that served as substrate for the diphtheria toxin; in this negative reaction they resemble eubacteria^{5,16}. Archaeobacteria and eukaryotes, on the other hand, serving as a control, give significant diphtheria toxin-dependent protein labelling.

While it is clear that *Planctomyces* and relatives are not related to archaeobacteria, the rRNA analyses do not indicate their status as a third line of descent of prokaryotes independent of those of archaeobacteria and eubacteria. Our data indicate that the

Table 2 Diphtheria toxin reaction with cell supernatants from budding, peptidoglycan-less eubacteria (I), peptidoglycan-containing eubacteria (II), archaeobacteria (III) and eukaryotes (IV)

		TCA-precipitable radioactivity (pmol NAD per mg supernatant protein)	
		With toxin	Without toxin
I	Strain IFAM 1310	0.23	0.22
	Strain IFAM 1317	0.16	0.11
II	<i>Escherichia coli</i>	0.20	0.15
	<i>Hyphomicrobium</i> sp.	0.22	0.16
III	<i>Methanococcus vannielii</i>	32.95	0.25
	<i>Thermoplasma acidophilum</i>	15.60	0.34
IV	Wheat germ	31.84	0.52
	Calf liver	18.81	0.36

The reaction, containing 10 μ l toxin solution (1.3 μ g protein nitrogen), 10 μ l NAD (adenine ¹⁴C, 277 Ci mol⁻¹, 75 pmol) and 50 μ l 150,000g supernatant containing 1–4 mg protein, was carried out at 37 °C for 30 min, and trichloroacetic acid (TCA)-precipitable radioactivity measured.

peptidoglycan-less bacteria have to be considered as eubacteria, with average S_{AB} values between *Planctomyces* and relatives and the eubacteria, being lower (0.15) than those found in the various lines of descent of eubacteria (0.22)^{15,17}. The exact phylogenetic position, however, cannot yet be determined, since it is not known whether the 16S rRNA cistrons of *Planctomyces*- and *Pasteuria*-like strains have evolved at the same rate as those of eubacteria, which, with certain exceptions^{18,19}, appear isochronic. If one assumes that the evolutionary rate of the strains investigated differs from that of the majority of eubacteria, the lack of peptidoglycan and of certain universal oligonucleotides present in the 16S rRNA of isochronically evolving eubacteria^{19,20} (that is, CACAAG, CUAACG, AAUACG, UUCCCG, AUACCCUG, AUUCCUACG) can be taken as positive evidence for this theory. In this case, the phylogenetic position of *Planctomyces* and relatives is within the main radiation of eubacterial species, either representing an individual line of descent or specifically related to members of one of its lines (as, for example, the fast-evolving mycoplasmas and relatives were found to be related to certain strains of *Clostridium*¹⁸).

However, a degenerate evolution of the budding, non-prosthecate and peptidoglycan-less eubacteria is not proven. The possibility therefore remains that these organisms represent the descendants of an ancient group of eubacteria that branched off immediately after the divergence of the 'ur-eubacteria' from

Table 1 Binary comparison among the 16S rRNA catalogues (S_{AB} values)

	IFAM 1310	IFAM 1319	IFAM 1317
Strain resembling <i>Pasteuria ramosa sensu</i> Staley ²²			
IFAM 1310	—	0.70	0.30
IFAM 1319	0.70	—	0.32
Strain resembling <i>Planctomyces maris</i>			
IFAM 1317	0.30	0.32	—
Eubacteria			
Purple bacteria and their non-phototrophic relatives*	0.06–0.11	0.06–0.15	0.06–0.15
Cyanobacteria*	0.09–0.15	0.10–0.16	0.09–0.16
<i>Spirochaeta</i> †	0.08–0.14	0.07–0.11	0.11–0.16
<i>Chloroflexus</i> / <i>Herpetosiphon</i> group†	0.07–0.08	0.07–0.10	0.11–0.16
<i>Cytophaga</i> / <i>Flavobacterium</i> group†	0.08–0.13	0.09–0.12	0.13–0.18
Mycoplasmas and relatives*	0.07–0.10	0.07–0.09	0.09–0.16
<i>Deinococcus</i> *	0.09–0.12	0.09–0.13	0.13–0.17
Clostridia and relatives*	0.08–0.17	0.09–0.15	0.11–0.18
Actinomycetes and relatives*	0.08–0.15	0.08–0.15	0.10–0.21
Archaeobacteria			
Methanogenic bacteria*	0.06–0.12	0.04–0.10	0.06–0.12
<i>Thermoplasma acidophilum</i> *	0.08	0.07	0.07
<i>Sulfolobus</i> and related taxa†	0.06–0.08	0.06–0.08	0.05–0.08

* The oligonucleotide catalogues of the reference strains have been published (see ref. 20).

† Unpublished data from the groups of C. R. Woese (University of Illinois, Urbana) and E.S.

the universal common ancestor of extant life, the 'progenote'²¹, and before the main radiation of eubacterial lines of descent^{17,19}. According to this theory the lack of certain universal oligonucleotides in the 16S rRNA catalogues implies not a loss due to high mutation rate, but rather that these universal eubacterial oligonucleotides evolved later in the evolution of eubacteria, after the ancestor of *Planctomyces* and relatives had already branched off. The eubacterial universal oligonucleotides missing in the catalogues of *Planctomyces* and relatives are also missing in archaeobacteria which diverged even earlier than did the budding, peptidoglycan-less eubacteria. Likewise, the lack of the peptidoglycan moiety reflects a primitive state of eubacterial cell walls, with the 'invention' of peptidoglycan following at a later stage in the evolution of eubacteria.

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Possible artefactual basis for apparent bacterial growth at 250 °C

Jonathan D. Trent, Roger A. Chastain & A. Aristides Yayanos

Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093, USA

Baross and Deming¹ reported that thermophilic marine bacteria isolated from the vicinity of a submarine hot-spring grow at temperatures up to at least 250 °C. They did not, however, conduct the appropriate control experiments to eliminate the possibilities of chemical artefacts or contamination. Here, in experiments using the same growth medium, the same temperature and pressure apparatus² and the same sampling and analytical procedures, we report results nearly identical to theirs. We conclude that their evidence indicating bacterial growth at 250 °C may be due to artefacts produced in the medium and to contaminants introduced during sample processing.

The 'black smoker' hydrothermal vent, discovered at a depth of 2,650 m at 21° N on the East Pacific Rise, releases 350 °C water into the ocean³. Water remains liquid at this temperature due to hydrostatic pressure. It has been suggested that the absence of liquid water, rather than high temperature *per se*, limits bacterial growth¹. This idea has been logically extended to predict that extreme thermophiles could inhabit submarine hydrothermal vents⁴.

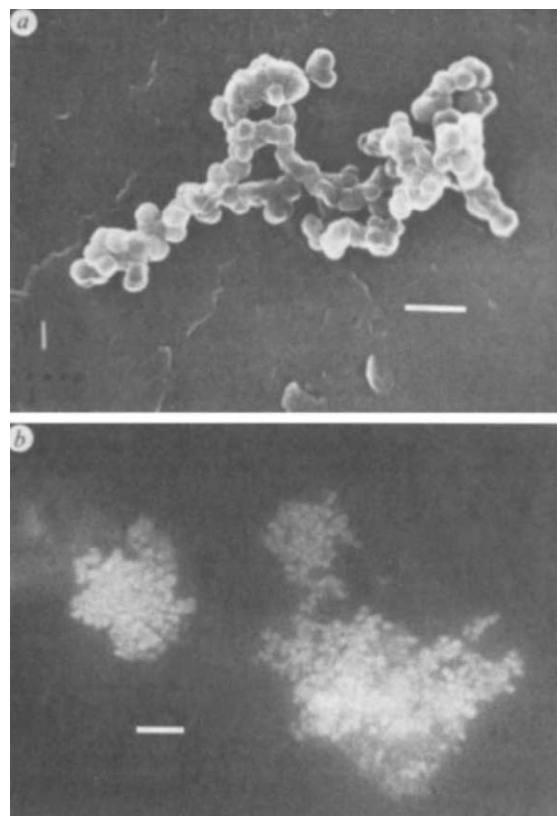


Fig. 1 *a*, Scanning electron micrograph of particles resembling bacteria from uninoculated medium after 4 h at 250 °C and 265 atm pressure. Scale bar, 1 μm. *b*, These particles are not DAPI-stained but autofluoresce, giving them some resemblance to DAPI-stained bacteria. Scale bar, 3 μm.

Methods: Experiments were conducted in a high-temperature and high-pressure apparatus². The temperature of this system at pressure is monitored by a thermocouple and a gauge thermometer inserted into the wall of the pressure chamber. The temperature inside the chamber was 238 ± 2 °C when the wall temperature read 249 ± 2 °C. Samples were taken before the medium was heated, when it reached maximum temperature, and then at approximately 1 h intervals for 5-6 h. Samples were fixed with 2% E.M.-grade glutaraldehyde and stored at 4 °C until analysed. For epifluorescent microscopy, glutaraldehyde-fixed samples were transferred in duplicate to sterile tubes. One subsample was untreated and used to measure autofluorescence and the other was stained with DAPI at a final concentration of $0.02 \mu\text{g ml}^{-1}$ for 1-3 h. Between 0.01 and 1.0 ml of sample was transferred into 5 ml of diluent (synthetic seawater with 0.25% formalin and 0.05% azide) and filtered onto 0.2-μm Nucleopore filters stained with Irgalan black. Over 200 objects were counted for each sample.

Baross and Deming's experiments were conducted using apparatus in this laboratory and their samples were analysed at Oregon State University. Their strongest evidence for the existence of 'black smoker' bacteria was based on growth curves, protein concentrations and transmission electron micrographs. Experiments were done at 265 atm pressure and 150, 200, 250 and 300° Celsius. An uninoculated control was done at 200 °C but no data are given. Controls were not done at any other temperature. Only at 250 °C is there a reasonable correlation between increases in cell concentrations and protein, and transmission electron micrographs of cells were from that temperature.

We requested a culture from Baross to verify their results independently. Although we did not receive the culture, we conducted experiments with uninoculated media and with media inoculated with a non-thermophilic marine bacterium, *Vibrio harveyi*. These experiments were designed to look for artefacts arising in the medium itself or in medium supplemented with cellular material.

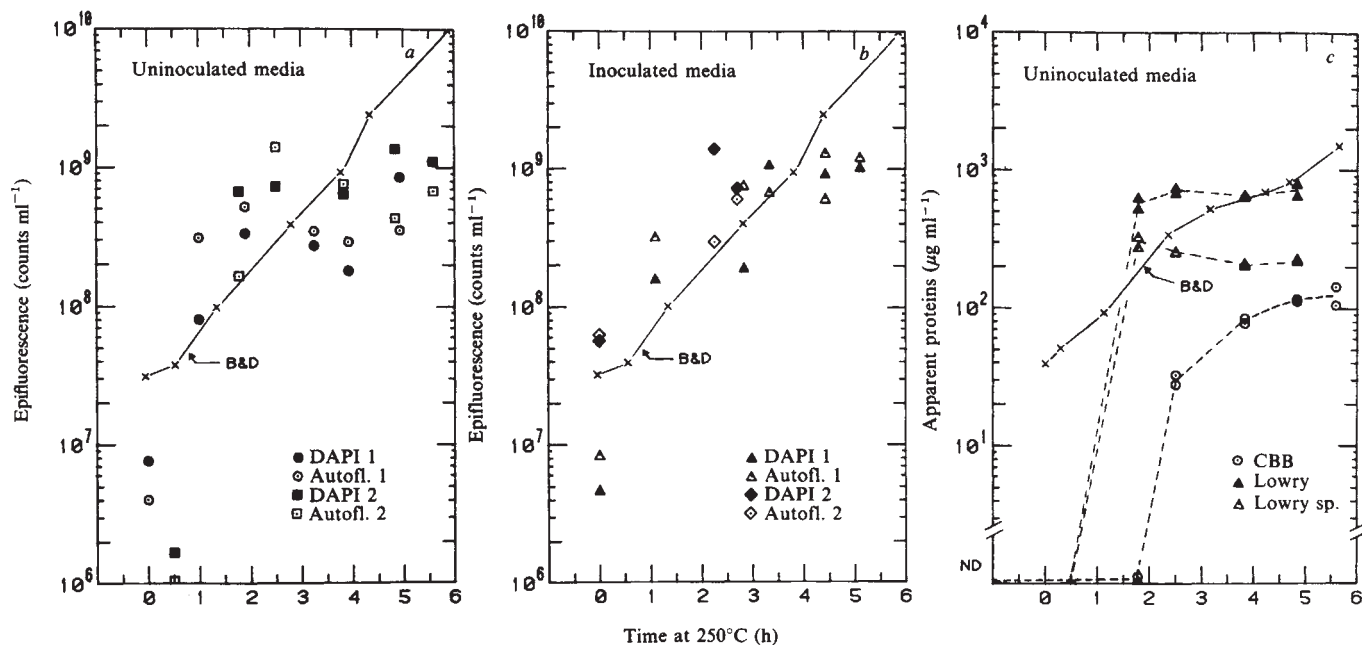


Fig. 2 *a*, In both experiments 1 and 2, the addition of DAPI did not increase counts above autofluorescence, indicating that DAPI staining did not occur. The solid curve labelled B & D shows the data reported by Baross and Deming. *b*, Experiments in which bacteria were added to media gave the same results as uninoculated media. The solid curve labelled B & D shows Baross and Deming's data. *c*, Before heating there is no measurable protein in the media using either the Coomassie brilliant blue (CBB) or the Folin-Lowry (Lowry) techniques. After heating to 250 °C for 1 or 2 h, colour develops, suggesting the presence of proteins. Part of the absorption in the Lowry technique is due to the presence of a precipitate; centrifuging samples to remove turbidity decreases absorption (Lowry sp.). The solid curve labelled B & D is from the original paper by Baross and Deming¹. 'ND' signifies not detected.

Methods: The medium is described as 'minimal salts' (MS) supplemented with: *a*, 0.03% (NH₄)₂SO₄; *b*, 0.001% Ca(NO₃)₂; *c*, 0.001% Bacto-yeast extract (Difco); *d*, 0.3% HEPES buffer (Sigma); *e*, 0.2% Na₂S₂O₃ · 5H₂O; *f*, 0.02% MnSO₄ · H₂O; *g*, 0.001% FeSO₄ · 7H₂O; *h*, 0.05% Na₂HPO₄; *i*, 0.5% NaHCO₃; *j*, trace element mix¹². Stock media consisted of seven solutions: minimal salts plus *a-d*, made as one solution at 2 times concentration; *e-i*, made as five separate solutions at 10 times concentrations; and *j*, made at the concentration at which it is used. All stock media were made with Nanopure water, stored at room temperature and except for *g*, which was filter sterilized, all were sterilized by autoclaving. For inoculated controls, 1 ml of stationary phase *V. harveyi* (~5 × 10⁸ cells) was centrifuged, rinsed with 1 ml of medium, re-centrifuged and suspended in the experimental medium. Protein concentrations in glutaraldehyde-fixed samples were measured using the methods reported by Baross and Deming¹. Aliquots of 0.5 ml were centrifuged at 13,000g for 1.5 min at 25 °C. The supernatant was aspirated and the pellet was precipitated by addition of 0.5 ml of cold 5% TCA for 1 h on ice. The sample was repelleted by centrifugation at 13,000g, 1 min at 4 °C; the precipitate was washed with cold 5% TCA as above, and pelleted a third time. The final pellet was solubilized with 200 μl of 1 M NaOH, and the pH adjusted to ~7 with 200 μl of HCl. The data reported in *c* are from the above method but variations on this method included direct protein measurements on unprocessed samples, TCA precipitation before initial centrifugation and no final addition of HCl to adjust pH. All procedures gave some colour development with heated media, suggesting that the Folin-Lowry and the Coomassie techniques should be used with caution, or that other protein assays should be used to evaluate these samples.

Our results showed no significant differences between uninoculated media and *V. harveyi* inoculated media at high temperature and pressure. A flocculent, white precipitate due to the mixing of manganese sulphate, sodium phosphate and sodium bicarbonate was present in the unheated media. This precipitate contained no epifluorescent particles above background and no measurable proteins. The amount of precipitate in the media decreased during heating and changed consistency from an amorphous floc to distinct micrometre-sized spheres (Fig. 1*a*). These spheres resembled bacteria in size and shape and were seen alone or in clumps (Fig. 1*a, b*).

For their growth curves, Baross and Deming measured changes in particles stainable with 4',6'-diamidino-2-phenylindole (DAPI), a DNA-specific stain routinely used to count bacteria⁵. The spheres we observed in heated media were autofluorescent and were not DAPI stained (Fig. 2*a, b*). Baross and Deming report observations of autofluorescence in their samples but they attribute it to methanogenic bacteria.

In our experiments the concentration of spheres increased for the first 2 or 3 h then stabilized at ~10⁹ per ml. In Baross and Deming's experiment at 250 °C, particles reached a concentration of 10¹⁰ per ml (Fig. 2*a, b*, curve B & D). We think this difference between our 'controls' and their experiment is not significant given the variability we observed due to mixing, sampling and counting.

Samples were processed for protein using the procedures described by Baross and Deming. Protein concentrations were

measured using the Folin-Lowry and the Coomassie brilliant blue techniques⁶. We found that heated media changed with time and gave positive readings in both protein assays (Fig. 2*c*). A number of non-protein substances have been found to interfere with the Folin-Lowry⁷ and Coomassie brilliant blue⁸ assays. We do not know the reason for the positive protein readings in our heated media.

Apparent protein concentration, measured with the Lowry technique, reached a plateau shortly after the media reached maximum temperature. Subtracting our apparent protein 'control' values from Baross and Deming's 250 °C 'bacteria' values (Fig. 2*c*, curve B & D), leaves only one data point above background at the end of their experiment. As in the case of epifluorescent microscopy, the difference between our data and theirs may be attributed to differences in sampling procedures and processing. For example, if precipitates are not removed by centrifugation or otherwise corrected for, apparent protein concentrations are significantly higher (compare Lowry and Lowry sp., Fig. 2*c*).

Baross and Deming present transmission electron micrographs of bacterial cells kept at 250 °C for 7 h. We used two techniques to prepare samples for transmission electron microscopy. Both methods resulted in satisfactory fixation of *V. harveyi* in the unheated medium (Fig. 3*a*). In heated media, the first method revealed the formation of micrometre-sized spheres that with few exceptions did not resemble cells (Fig. 3*b*). Using the second method (A. Soeldner, personal communication),

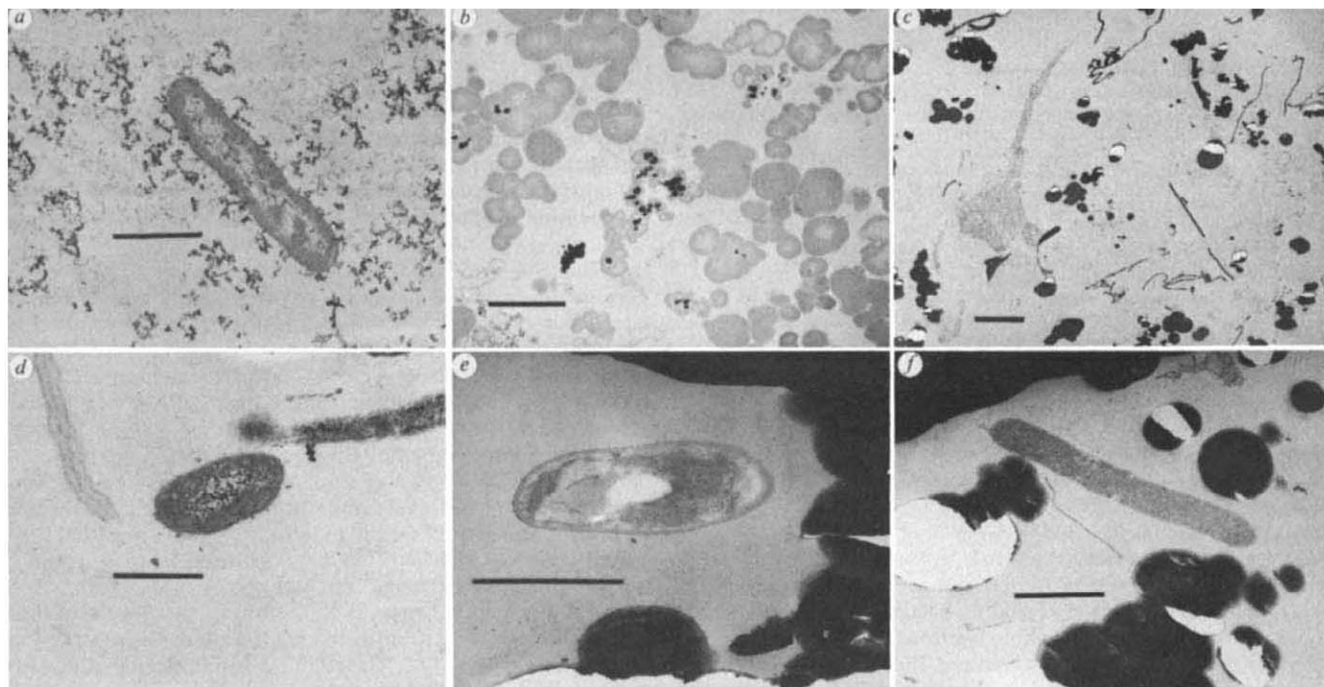


Fig. 3 *a*, A transmission electron micrograph of unheated medium inoculated with the non-thermophilic bacterium *V. harveyi*. The cell is surrounded by the precipitate present in the starting medium. Scale bar, 1 μ m. *b*, After heating to 250 °C at 265 atm pressure, all *V. harveyi* cells disappeared. Using an embedding technique with filtered agar, only micrometre-sized spheres were found in a sample after 4 h at temperature and pressure. Scale bar, 3 μ m. *c*, The same sample shown in *b* processed using the embedding procedure used by Baross and Deming, shows the same spheres more darkly stained and a variety of other objects including cells. Scale bar, 3 μ m. *d-f*, Examples of cells and cell-like objects found in thin sections from uninoculated media heated to 250 °C for 4 h and processed using the same transmission electron microscopic procedure used by Baross and Deming. Scale bars: *d*, 0.5 μ m; *e*, *f*, 1 μ m.

Methods: Immediately after sampling, specimens were fixed for electron microscopy by adding glutaraldehyde to a final concentration of 2%. Fixed material was washed with 0.2 M cacodylate buffer (pH 7.8) and postfixed with 1% OsO₄ in buffer. In one procedure fixed samples were embedded in 0.22 μ m filtered 2% Nobel agar (Difco) in buffer and dehydrated in ethanol. In the other procedure samples were embedded in unfiltered Ionagar (Oxoid no. 2) in buffer, dehydrated in acetone and stained with uranyl acetate before final dehydration (70% step). All specimens were infiltrated with Spurr resin. Silver-grey thin sections were post-stained with 4% uranyl acetate and 0.1% lead citrate.

which was used by Baross and Deming, we found many objects that resembled cells (Fig. 3*c-f*). The critical difference between the two methods was the use of 0.2- μ m filtered embedding agar in the first method and the use of unfiltered agar in the other method. It thus seems likely that the cells seen by Baross and Deming in samples incubated at 250 °C and 265 atm did not survive exposure to these conditions but were contaminants added to the samples after they were cooled and decompressed.

The production of methane, hydrogen and carbon monoxide and the presence of protein amino acids are also presented as evidence for black smoker bacteria. Gas production in Baross and Deming's experiments was measured after 3 h at 300 °C. No gas data are reported from 250 °C. Their uninoculated media control was done at 200 °C rather than 300 °C and reportedly did not produce gases. We did not analyse our 'control' samples for gas production.

We determined protein amino acids on three of our samples using Baross and Deming's procedures. One sample was from uninoculated and unheated medium, a second sample was from medium uninoculated and heated for 4 h at 250 °C, and a third sample was from medium inoculated with *V. harveyi* and heated for 4.5 h at 250 °C. These samples were analysed by the Amino Acid Dating Laboratory at Scripps Institution of Oceanography. A total of 200 nmol ml⁻¹ (predominantly serine, glutamic acid, glycine and aspartic acid) was measured in the trichloroacetic acid (TCA)-precipitable fraction of our unheated, uninoculated sample. Other amino acids were present at the detection limits of the analytical facility (~1 nm). In samples of both the uninoculated and inoculated media that were heated to 250 °C, total amino acids in the TCA-precipitable fraction were 120 and 80 nmol ml⁻¹, respectively, with the same four amino acids found in unheated samples predominating. We think the levels of amino acids measured in our control samples were due to

contamination in the pressure apparatus or from reagents used in sample processing.

Amino acid data reported in Baross and Deming's Table 1 are fractions (addition of their data totals 1.0002). Their caption states that concentrations of amino acids can be derived from their total colorimetrically determined protein data minus 25% for unknown peaks. Since we have shown that their colorimetric protein determinations are suspect, it is not clear what concentrations of amino acids are actually present in their samples, or if they are greater than the levels of contamination we found in uninoculated media. They report a number of amino acids, such as serine and threonine, which are known to be unstable at elevated temperatures⁹. Contamination is a significant problem when measuring trace amounts of amino acids^{10,11}. Since Baross and Deming's amino acid data are based on a single determination, without controls, we do not consider them good evidence for the existence of black smoker bacteria.

In Baross and Deming's experiments only their results from 250 °C were suggestive of bacterial growth. There were no controls done at this temperature and thus no way to eliminate artefacts. The experiments reported here indicate that artefacts are produced in media heated to 250 °C at 265 atm pressure in the experimental system used². These artefacts cast some serious doubts on their reported growth curves and protein data. Contamination and abiotic micrometre-sized spheres could account for the micrographs of 'cells' Baross and Deming reported from their 250 °C samples. Data on gas production and amino acids are not presented in sufficient detail to be seriously considered supporting evidence for the existence of black smoker bacteria.

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Reply from John A. Baross* and Jody W. Deming†

* School of Oceanography, Oregon State University, Corvallis, Oregon 97331, USA

† Chesapeake Bay Institute and Department of Biology, The Johns Hopkins University, Shady Side, Maryland 20764, USA

The conclusion of Trent *et al.* that no bacterial growth occurred in our experiments¹ is not tenable because of crucial differences between the results of our work and theirs, and because of additional data not addressed by these authors. If the scores of morphologically diverse bacteria we observed in thin sections of a 250 °C sample were contaminants, they would have appeared in the 300 °C sample also reported¹, since both samples were processed simultaneously using the same reagents. Such was not the case. Recent amino acid analyses² from our original experiments¹ indicate that at 250 °C the total amino acid content of particulate protein increased from 1.6 to 145 µg ml⁻¹ in 5.4 h, a doubling time of approximately 1 h. The same doubling time was calculated for concentrations of each of the 15 amino acids (including serine and threonine) detected by the standard methods we used^{1,2}. We continue to work with the original culture of black smoker bacteria, as well as the viable culture recovered from the titanium syringe upon termination of the 250 °C experiment, and one of us will dive with the French in March 1984 to search for similar bacteria in unexplored smokers at 13° N.

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Role of an upstream regulatory element in leucine repression of the *Saccharomyces cerevisiae leu2* gene

Alfonso Martinez-Arias*‡, H. Joseph Yost† & Malcolm J. Casadaban*†

* Department of Biophysics and Theoretical Biology, and † Committee on Genetics, University of Chicago, Chicago, Illinois 60637, USA

The expression of a number of eukaryotic genes has been shown to involve at least two sequences located upstream of the actual transcription unit^{1–7}: one of these sequences, centred on a widely conserved TATAAT sequence, is thought to be involved in determining the precise site of initiation of transcription^{1,8}; the other has a gene-specific sequence, can function at a variable distance upstream of the initiation site^{3,9}, and is involved in the regulation of transcription^{3,4,7,10}. By constructing β -galactosidase gene fusions, to facilitate measuring gene expression *in vivo*, we have now defined a *cis*-acting regulatory element

of the *Saccharomyces cerevisiae leu2* gene. This element is located within a 280 base pair (bp) fragment which occurs 125 bp upstream of the *leu2* translation initiation codon and which contains a short G + C-rich palindromic sequence. A fragment of the *Escherichia coli* transposable element Tn9 which contains a similar palindromic sequence can functionally replace the natural *leu2* regulatory element. Our results are contrary to previous speculations that the *leu2* gene is regulated by an attenuation mechanism^{11,12}.

Biochemical studies of the *leu2* locus of *S. cerevisiae*, which encodes β -isopropylmalate dehydrogenase, an enzyme of the leucine biosynthetic pathway, have indicated that enzymatic activity is repressed when cells are grown in the presence of leucine and threonine¹³. DNA sequence analysis of the region upstream of *leu2*¹² has revealed the complex arrangement (Fig. 1) of a Ty1 transposable element, a leucyl tRNA gene and an open reading frame with the potential for encoding a 24 amino acid polypeptide containing 6 leucine residues. This led to speculation about the regulatory mechanisms that may be operating at this locus^{11,12}. To investigate further the nature of this regulation an *XhoI*–*BstEII* fragment, which extends from the Ty1 δ element to the 13th *leu2* codon, was fused *in vitro* to the *E. coli* β -galactosidase gene on the vector pMC1790^{14,15}, as described in Fig. 1. Comparable fusions to the β -galactosidase gene have been used to study the regulated expression of several other genes in yeast^{16–18}. The resulting fusion plasmid pMC1876 expresses a level of β -galactosidase in transformed¹⁹ yeast cells that is repressed fivefold when the cells are grown in the presence of 1 mM leucine and 1 mM threonine (Table 1).

The *leu2* control region in the fusion plasmid was dissected as illustrated in Fig. 2 and assayed as summarized in Tables 1 and 2. A deletion from the upstream side extending to the middle of the tRNA^{leu} gene sequence ($\Delta 1$) results in a small increase in β -galactosidase expression but no qualitative changes in the pattern of regulation. Total removal of tRNA^{leu} gene sequences in this region ($\Delta 8$) leads to a further increase in β -galactosidase expression. This suggests that the tRNA gene is not directly involved in the regulation of this locus, although its presence does exert a small effect on *leu2* expression, perhaps due to steric interactions between factors bound to these two closely linked genes²⁰. Removal of sequences up to the *HincII* site located 125 bp upstream of the *leu2* translation initiation codon ($\Delta 2$) results in a 100-fold decrease in β -galactosidase activity and the loss of leucine-specific regulation. This indicates that regions important for regulation exist between the tRNA gene and the *HincII* site. Moreover, as the $\Delta 2$ deletion does not remove the major transcription initiation site (A. Andreadis and P. Schimmel, personal communication) the regulation of this locus presumably takes place primarily at the level of transcription.

To explore this further, we exchanged the DNA fragment between the *HincII* site and the *BstEII* site in $\Delta 1$ with a 250 bp piece of DNA containing the transcription and translation initiation regions from the *cyc1* gene of *S. cerevisiae* fused to the β -galactosidase gene^{17,21}. When this fusion, which has a hybrid promoter (HY $\Delta 1$), was assayed in yeast, β -galactosidase activity was regulated by leucine and threonine (Table 2). This fusion does not respond to other amino acids in the culture medium nor to catabolite repression, which is the normal regulatory pattern of the *cyc1* gene. This indicates that the *leu2* regulatory element is located upstream of the *HincII* site and well before the *leu2* coding region. It is interesting, however, that the levels of β -galactosidase expressed by HY $\Delta 1$ are lower than those of the wild-type *leu2* promoter, and that the ratio of the levels of expression in repressing and non-repressing conditions is larger. This might reflect differences in translation or stability of the hybrid proteins from the fusions, or it might reflect a requirement for a precise interaction between the upstream regulator and the initiation site-proximal sequences, which is altered in the hybrid promoter.

In the DNA sequence¹² between the $\Delta 8$ and the $\Delta 2$ end points, we noted the presence of a short palindromic sequence

‡ Present address: MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.

Table 1 Repression of *leu2* expression

Plasmid*	leu2 activity†			
	No amino acids	Leu + Thr	His	No amino acids: Leu + Thr
pMC1876	640	140	720	4.6
Δ1	940	320	1,000	3.0
Δ8	1,400	390	1,400	3.6
Δ2	12	13	13	0.9
Δ26	19	20	ND	0.9
InΔ1	930	410	ND	2.3

* Yeast strain M12B (α , *trp1*-289, *ura3*-52, obtained from K. Struhl and R. Davis) cells were transformed¹⁹ with the indicated plasmids. The cells were grown in SD minimal media²⁶ supplemented with uracil or tryptophan (40 μ g ml⁻¹) and the indicated amino acids (1 mM each).

† The *leu2* assays were performed in triplicate on exponentially growing cells at a concentration of 10⁷ cells per ml as described previously¹⁵. Units of activity are nmoles *o*-nitro-phenyl- β -D-galactoside (ONPG) cleaved per min per mg of total protein in the extract. The different levels are not due to changes in the segregation properties of the plasmids (data not shown). ND, not determined.

(5' TGAGAGGCCGGAACCGGCTTTTCA 3') containing two *Hpa*II sites (CCGG) and centred 192 bp from the *leu2* translation initiation codon. Short repeats similar to this have been implicated in the transcriptional regulation of several eukaryotic loci^{3,7,22}. To test the involvement of this sequence in the regulation of *leu2* expression, the 6 bp of DNA between the two *Hpa*II sites were deleted (Δ 26, Fig. 2) from the fusion (Δ 8) lacking the tRNA^{Leu} gene. When placed into yeast, this deletion-fusion displayed a very low constitutive β -galactosidase activity and indicates that this G+C-rich palindrome lies within a region important for regulation as well as expression. This small deletion (Δ 26) lowers dramatically the basal levels of β -galactosidase expression (Table 1); this might be due to the loss of the functional entry site of RNA polymerase to the *leu2* locus or to the appearance of abnormally initiated transcripts. The assay used in these experiments cannot distinguish between these possibilities. In a second experiment the DNA region between the *Hpa*I and *Hinc*II sites was inverted (InΔ1, Fig. 2). This inversion moves the palindromic sequence 150 bp further upstream of the transcription site. In yeast, this fusion yielded levels of β -galactosidase comparable to the wild-type fusion, but with only a twofold repression by leucine plus threonine (Table 1). These results are consistent with a possible role of this symmetric structure in the regulation of expression at the *leu2* gene.

In an additional experiment aimed at identifying sequences capable of substituting for the *leu2* upstream modulator element by restoring leucine regulated β -galactosidase expression to plasmid Δ 2, various DNA sequences were inserted into the *Eco*RI site at the left end of Δ 2 *leu2* DNA. One fragment, derived from Tn9²³, restored expression comparable to Δ 1 with a twofold leucine plus threonine regulation (data not shown). Examination of the sequence of this fragment²⁴ revealed a 5' GGTGCGTACCGGGTT 3' sequence related to the *leu2*

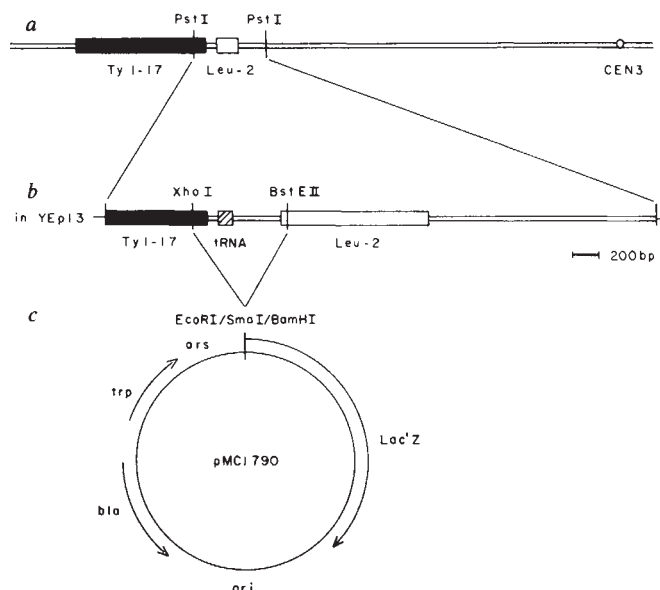


Fig. 1 a, Location of the *leu2* locus on yeast chromosome III. b, The *Pst*I fragment of the original *leu2* gene cloned in YEpl3²⁷. The *leu2* gene is transcribed from left to right¹⁵. There is a *Ty1* element, a tRNA^{Leu} gene and an open reading frame (ORF) upstream from the *leu2* coding region¹². c, The fusion plasmid pMC1876 was formed by inserting a blunt-ended *Xho*I-*Bst*EII fragment from YEpl3 into the *Sma*I site of vector pMC1790¹³, as described previously¹⁴. This construction has the first 13 *leu2* codons fused in phase to the eighth codon of *lacZ*, so that a hybrid protein with β -galactosidase activity is encoded. *E. coli* strain MC1066¹⁴ carrying plasmid pMC1876 is ampicillin resistant, Trp⁺ and expresses β -galactosidase activity detectable on M63 Xgal medium²⁸. Note that inserts into the *Sma*I site of pMC1790 are flanked on the left by an *Eco*RI site and on the right by a *Bam*HI site.

repeat region located 170 bp from the *leu2* translation initiation site. Although this region does not contain a perfect palindrome, it embraces a core sequence quite homologous to the one present in *leu2* (see above). This control sequence also appears in a similar position in the promoter of *leu1* (Yun-Pung Hsu and P. Schimmel, personal communication), another leucine biosynthetic pathway gene which is coordinately regulated with *leu2*²⁵. Further mutational analysis of the *leu2* control region will be necessary to determine precisely which bases are essential for leucine regulation.

The results presented here exclude any major *cis*-acting regulatory role for the open reading frame upstream of the *leu2* gene. Plasmid HYΔ1 lacks the 3' end of the open reading frame and still shows *leu2* regulation; on plasmid InΔ1 the open reading frame is disrupted by inversion; and the Tn9 substitution lacks the 5' end of the open reading frame. Furthermore, small insertions and deletions around the *Hinc*II site within the open reading frame do not eliminate regulation even in the absence

Table 2 Repression of hybrid promoter *leu2-cyc1*

Plasmid	leu2 activity				Raffinose: glucose
	No amino acids	Leu + Thr	His	Raffinose	
pMC1876Δ1	940	320	1,000	1,100	2.9
pLG669Δ292	4,000	5,200	ND	10,400	0.8
pMC1876HYΔ1	210	30	260	340	7.0
					1.6

The *cyc1-lacZ* fusion plasmid²¹ pLG669Δ292 contains a 300 bp fragment of the *cyc1* gene from *S. cerevisiae*, a 2 μ replicon, and a *ura3* gene as a selectable marker. Amino acids were added at 1 mM each. 2% raffinose was substituted for 2% glucose as the carbon source to determine the response of the fusions to catabolite repression.

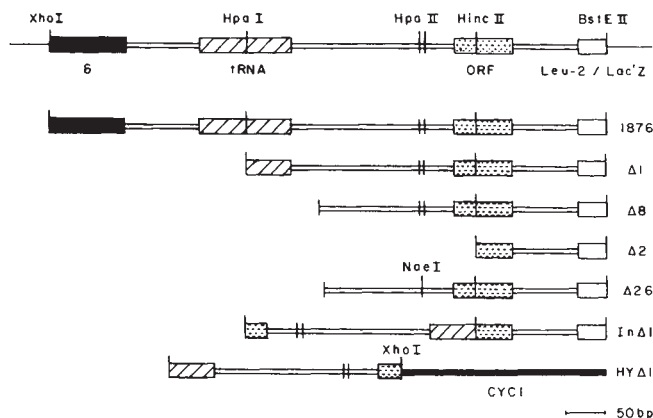


Fig. 2 Derivatives of pMC1876. The $\Delta 1$ and $\Delta 8$ fragments were obtained by cleaving pMC1876 on the left (*Xho*I site) with *Eco*RI, digestion with the exonuclease *Bal*31 followed by cleavage on the right (*Bst*EII site) with *Bam*HI and isolation in 4.5% polyacrylamide gels. $\Delta 2$ is a *Hinc*II–*Bam*HI fragment of pMC1876. The $\Delta 1$, $\Delta 8$ and $\Delta 2$ fragments were inserted into pMC1790 cleaved with *Sma*I and *Bam*HI. $\Delta 26$ is a 6 bp *Hpa*II deletion obtained by isolating the *Eco*RI–*Bam*HI fragment of $\Delta 8$, cleaving with *Hpa*II, and ligating the two large fragments from this reaction (eluted from a polyacrylamide gel) back into pMC1790 cleaved with *Eco*RI and *Bam*HI. This construction was verified by the observation that the deletion of the 6 bp between the *Hpa*II sites (5' GCCGGAACCGGC 3') creates a *Nae*I site (5' GCCGGC 3'). The hybrid *leu2*–*cyc1* promoter was formed by cleaving the derivative of $\Delta 1$, containing an *Eco*RI–*Sma*I–*Bam*HI linker at the *Hinc*II site, with *Sma*I and *Sac*I (a unique restriction site in the middle of *lacZ*) and ligating the purified vector fragment to a fragment containing the *cyc1* transcription and translation initiation sites from pLG669 Δ 292²¹ cleaved with *Xho*I, rendered blunt, and cleaved with *Sac*I. All clones were selected in *E. coli* strain MC1066 as ampicillin resistant, Trp⁺ colonies displaying β -galactosidase activity on M63 Xgal medium. Note that the fusion points in *lacZ* of the *cyc1* and the *leu2* are different. DNA manipulations²⁹, strains and methods of selection used¹⁴ have been described previously. The end points of $\Delta 1$, $\Delta 8$ and $\Delta 2$, and the centre of the palindromic repeat are located 400 ± 10 , 310 ± 10 , 125 and 192 bp respectively before the *leu2* initiation codon.

of the chromosomal copy of the open reading frame at the *leu2* locus (unpublished results).

Mutations at upstream modulator elements, similar to our $\Delta 26$, affect both gene regulation and expression systems that have been investigated^{4,7,17}. This phenomenon has been interpreted as evidence that these sequences mediate positive regulatory mechanisms at these loci and thus may represent binding sites for specific proteins whose function is to open the chromatin structure locally, allowing RNA polymerase positioning. Alternatively these elements may be entry sites for the RNA polymerase destined to initiate transcription at the downstream TATA box.

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Expression and regulation of human low-density lipoprotein receptors in Chinese hamster ovary cells

Robert D. Sege, Karen Kozarsky, David L. Nelson & Monty Krieger*

Department of Biology, Whitaker College of Health Sciences, Technology and Management, and the Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Low-density lipoprotein (LDL), the major cholesterol transport component of human plasma, delivers cholesterol to mammalian cells via the LDL pathway of receptor-mediated endocytosis¹. LDL receptor activity and cholesterol biosynthesis are coordinately regulated by cholesterol-mediated feedback suppression^{2–4}. We have developed methods for the isolation of mutant^{5,6} and revertant (M.K., unpublished data) Chinese hamster ovary (CHO) cells having alterations in cholesterol biosynthesis and in the receptor-mediated endocytosis of LDL. The defective locus of one LDL receptor-negative CHO mutant (clone 7a-1)⁶ is apparently the structural gene for the LDL receptor (D. Kingsley, M. Segal and M.K., unpublished data). Here we have transfected 7a-1 cells with human DNA and selected colonies which synthesize functional human LDL receptors whose expression is regulated normally. This selection may prove useful for cloning genes required for the receptor-mediated endocytosis of LDL and other ligands and thus for elucidating the molecular defects responsible for familial hypercholesterolaemia, one of the most common genetic diseases in humans⁷.

Human DNA was introduced into the LDL receptor-negative clone 7a-1 by the CaPO₄ method^{8,9} and cells were selected in MeLoCo medium as described in Fig. 1 legend. In this selective medium the cells die from cholesterol deprivation unless they can obtain cholesterol by the endocytosis of LDL^{5,6,10,11}. Colonies survived continuous selection in MeLoCo medium at a frequency of 2×10^{-7} (6 colonies per 3×10^7 cells). This frequency of survival was ~1,000-fold lower than that obtained when the neomycin analogue G418 was used to select pSV2-neo transfected cells¹² (data not shown). Two of the colonies which survived MeLoCo selection, designated LET-1 and LET-2 (LDL endocytosis transfectants), were selected for further study. These colonies contained discrete DNA sequences homologous to human repetitive DNA (*Alu* family), whereas untreated 7a-1 cells did not (Fig. 1).

The expression of the LDL receptor pathway in LET-1 and LET-2 was compared with that in parental CHO and receptor-negative 7a-1 cells by measuring the specific cellular binding,

* To whom reprint requests should be addressed.

Fig. 1 Detection of human repetitive DNA sequences in CHO cells following transfection and selection. All cells were cultured in Ham's F-12 media supplemented with the indicated sera as described previously^{5,6,13}. DNA, isolated from LDL receptor-negative clone 7a-1 and transfected LET-1 and LET-2 cells, was digested with *EcoRI* and analysed by Southern blot^{28,29} hybridization with ³²P-labelled³⁰ cloned human *Alu* family (BLUR11)³¹ DNA. Kodak XAR-5 film was exposed at -70 °C for 20 h with a Cronex Lightning Plus screen (Dupont). LDL endocytosis transfectants (LETs) were obtained as follows: 7a-1 cells were transfected with human DNA using the CaPO₄ technique⁹ with minor modifications. DNA was extracted³² from portions of two human fetal livers which were pulverized in the presence of liquid nitrogen³³ and further purified by sequential digestion with ribonuclease A (Sigma) and proteinase K (Boehringer-Mannheim). 24 h before transfection, the cells were plated (7.5×10^5 cells per 100 mm plate) in 10% newborn calf serum (NCS) and the DNA was sheared three times through a 21-gauge needle (final length >45 kilobases (kb) by agarose electrophoresis and precipitated by overnight incubation with ethanol at -20 °C. The precipitate was dissolved ($30 \mu\text{g ml}^{-1}$) in HEPES-buffered saline (HBS), pH 7.1. The CaPO₄/DNA coprecipitate was allowed to form for 45 min at room temperature, and added (1.0 ml per dish) to cell monolayers from which medium had been removed. After incubation for 20 min at room temperature, fresh 10% NCS was added and the plates were incubated at 37 °C for 4.5 h. Cells were then treated with 15% glycerol in HBS; 2 days later, cells were replated (7.5×10^5 cells per 100 mm dish) in selective medium (3% newborn calf lipoprotein deficient serum (NC-LPDS) containing a mixture of mevalonate (238 μM), LDL ($2.8 \mu\text{g protein ml}^{-1}$) and compactin (38 μM), called MeLoCo)^{5,6,10,11}. The compactin inhibits synthesis of mevalonate and thus inhibits synthesis of steroidal and non-steroidal isoprenoids; the trace levels of mevalonate permit the synthesis of non-steroidal but not steroidal isoprenoids; and the LDL serves as a source of sterol for cells which express the LDL pathway. Most of the cells began to die within 3 days and macroscopic colonies of survivors were visible by eye after 13–17 days of selection. These colonies (LETs) were picked³⁴ and subsequently maintained in selective medium. An analogous selection relying on the reversal of nutritional deficiencies by receptor-mediated delivery of inorganic ions has been suggested³⁵.

uptake and degradation of ¹²⁵I-LDL ($10 \mu\text{g protein ml}^{-1}$)¹⁴. Relative degradation activities were: parental CHO, 100%; LET-1, 184%; LET-2, 93%; and 7a-1, 2%. The saturation kinetics of ¹²⁵I-LDL degradation by LET-1 (Fig. 2) and LET-2 (data not shown) cells exhibited high affinity and saturability, hallmarks of the classic LDL pathway¹⁴. The affinity of LET-1 cells for LDL at 37 °C (K_m $16 \mu\text{g protein ml}^{-1}$) was twice as strong as that of parental CHO cells (K_m $35 \mu\text{g protein ml}^{-1}$) and was essentially equal to that of human LDL receptors (K_m $17 \mu\text{g protein ml}^{-1}$)¹⁵. LET-1 bound twofold more LDL than CHO cells at 4 °C (data not shown), and the ratio of ¹²⁵I-LDL internalization and degradation to surface binding, that is, the efficiency of the LDL pathway¹⁶, in LET-1 cells (ratio 23) was essentially identical to that in CHO cells (ratio 24, data not shown).

These results suggested that LET cells survived selection because transfection and expression of the human LDL receptor gene complemented the mutation in clone 7a-1. Therefore, synthesis of the human LDL receptor (an ~160,000-molecular weight (MW) monomeric glycoprotein)¹⁷ was examined by immunoprecipitation and electrophoresis¹⁸. A previously described¹⁹ monoclonal antibody against bovine LDL receptor (IgG C7) cross-reacted with and inhibited LDL binding to human LDL receptors (Fig. 3a and ref. 19) but not to LDL receptors from CHO cells (Fig. 3b and ref. 19). No specific immunoprecipitable protein was detected in 7a-1 cells

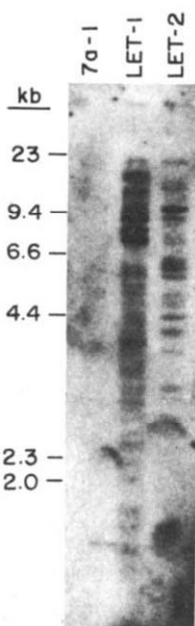


Table 1 Degradation of ¹²⁵I-LDL by LET-1 cells: inhibition by anti-human receptor antibody and regulation by LDL and sterols

	¹²⁵ I-LDL degradation (% of control)	
	Parental CHO cells	LET-1 cells
Expt a		
Additions to assay medium		
None	100	100
IgG C7 (190 nM)	104	49
IgG 2001 (190 nM)	121	117
Expt b		
Additions to growth medium		
None	100	100
LDL ($300 \mu\text{g protein ml}^{-1}$)	25	35
Cholesterol ($12.5 \mu\text{g ml}^{-1}$)	72	77
Cholesterol + 25-hydroxycholesterol ($0.5 \mu\text{g ml}^{-1}$)	24	39

Expt a: On day 0, parental CHO and LET-1 cells were plated in 1.5 ml of 3% NC-LPDS in 24-well plates (60,000 cells per well). On day 2, each monolayer was preincubated for 1 h with 0.5 ml of 3% NC-LPDS containing either no additions or 190 nM of anti-bovine (human) LDL receptor monoclonal antibody (IgG C7) or control antibody (IgG 2001). Each monolayer was re-fed with 5% H-LPDS containing the same additions plus $10 \mu\text{g protein ml}^{-1}$ of ¹²⁵I-LDL (247 c.p.m. ng⁻¹). After incubation at 37 °C for 5 h, the amount of ¹²⁵I-LDL degradation products released into the medium was determined. The values are expressed as a percentage of the high affinity values for the cells incubated in the absence of antibody. The 100% control values were: CHO, 1,795 ng per 5 h per mg; LET-1, 4,034 ng per 5 h per mg. **Expt b:** In a separate experiment, cells were plated on day 0 as described above. On day 1, the medium was replaced with 1 ml of 3% NC-LPDS containing the indicated additions of LDL, cholesterol in ethanol or cholesterol plus 25-hydroxycholesterol. On day 2 (23 h later), each monolayer was washed once with phosphate-buffered saline, then 0.5 ml of 5% H-LPDS containing $10 \mu\text{g protein ml}^{-1}$ of ¹²⁵I-LDL (446 c.p.m. per ng protein) was added and high affinity degradation was determined. The high affinity values are expressed as the percentage of the values for the same cells incubated with no additions. The 100% control values were: CHO, 1,293 ng per 5 h per mg; LET-1, 2,698 ng per 5 h per mg. The thrombin used to isolate H-LPDS was given by the Parke-Davis Division of Warner Lambert.

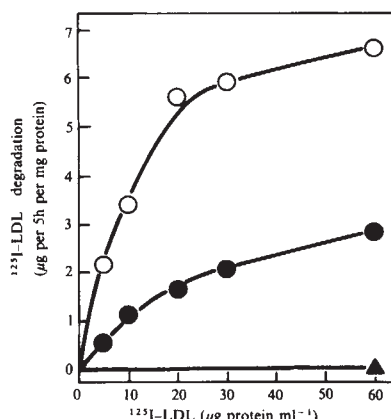
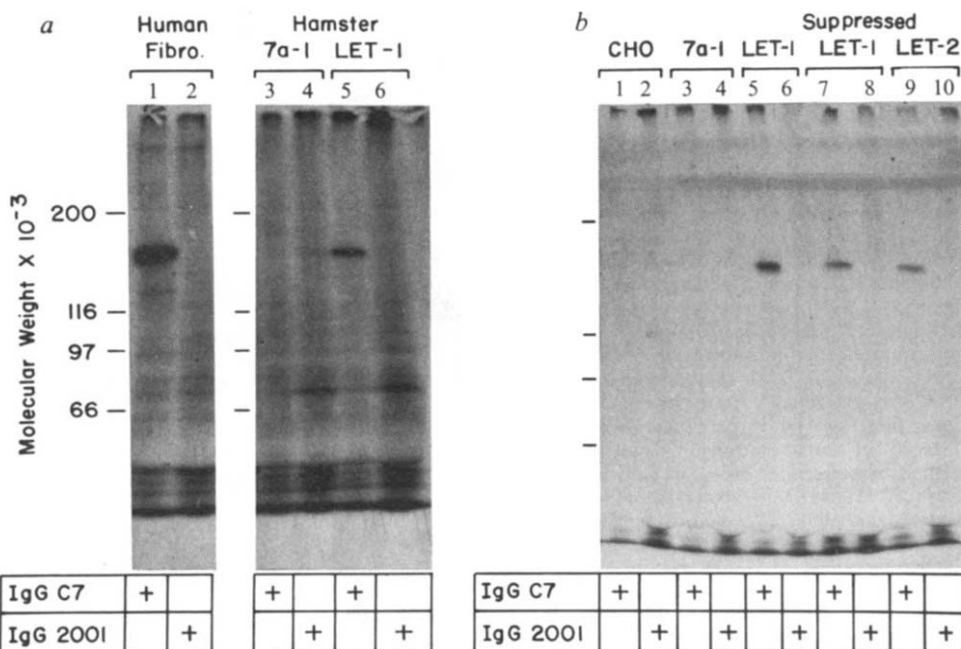


Fig. 2 Saturation kinetics for the degradation of ¹²⁵I-LDL at 37 °C by monolayers of parental CHO, LDL receptor-negative clone 7a-1 and LET-1 cells. On day 0, parental CHO (●), clone 7a-1 (▲) and LET-1 (○) cells were seeded in 1.5 ml of 3% NC-LPDS into 24-well plates (60,000 cells per well). On day 2, each monolayer received 0.5 ml of 5% human lipoprotein deficient serum (H-LPDS) containing the indicated amounts of ¹²⁵I-LDL (442 c.p.m. per ng of protein). After incubation at 37 °C for 5 h, the amounts of ¹²⁵I-LDL degradation products released into the media were determined as described previously¹³. Data are expressed as high affinity values for degradation.

(Fig. 3a, b). IgG C7 specifically precipitated one protein of MW ~160,000 from both LET-1 (Fig. 3a, b) and LET-2 (Fig. 3b). IgG C7 also specifically inhibited degradation of ¹²⁵I-LDL by LET-1 but not by parental CHO cells (Table 1, expt a). Thus, human LDL receptors in these hamster cells are respon-

Fig. 3 Immunochemical detection of human LDL receptors in transfected hamster (LET) cells: regulation of expression by LDL. *a*, On day 0, normal human diploid fibroblasts (GM3349)¹³ were plated in 3 ml of 10% fetal bovine serum in 60 mm dishes (50,000 cells per dish). On day 2, the monolayers were re-fed, then on day 5 they were washed and re-fed with 3 ml of 10% H-LPDS. In addition, LDL receptor-negative clone 7a-1 cells and human DNA transfectants of clone 7a-1 (LET-1) were plated on day 5 in 3 ml of 3% NC-LPDS (300,000 cells per 60 mm dish). On day 6, all monolayers were re-fed with either 10% H-LPDS (fibroblasts) or 3% NC-LPDS (7a-1 and LET-1 cells) prepared using methionine-free Ham's F-12 medium (K-C Biologicals) supplemented with 50 $\mu\text{Ci ml}^{-1}$ of ^{35}S -methionine (NEN) and 5 μM unlabelled methionine (Sigma). After 16 h, the monolayers were washed, lysed, and immunoprecipitated with either anti-bovine (and human) LDL receptor antibody (IgG C7: lanes 1, 3 and 5) or control antibody (IgG 2001: lanes 2, 4 and 6) as described¹⁸. Each lane represents immunoprecipitates derived from 6.4×10^7 (A) or 10^8 (B) c.p.m. of labelled cell extract. The immunoprecipitates were subjected to SDS-6% polyacrylamide gel electrophoresis³⁶ (7 mA per gel) for 18 h, impregnated with Autofluor (National Diagnostics), dried and subjected to autoradiography (Kodak XAR-5 film at -70°C for 7 days with a Cronex Lightning Plus screen). *b*, Parental CHO, 7a-1, LET-1 and LET-2 cells were plated on day 5 as described above except that the LET-1 monolayers were divided into two groups. The first group (lanes 5 and 6) was plated in 3% NC-LPDS as were the other cell strains (see above). The second group, 'suppressed LET-1' (lanes 7 and 8), was plated and labelled in 3% NC-LPDS containing LDL (300 $\mu\text{g protein ml}^{-1}$). The cells were processed for immunoprecipitation with IgG C7 or IgG 2001 as indicated, and for electrophoresis and fluorography (24 h exposure) as described above.



sible for the receptor-mediated endocytosis of LDL.

A distinctive feature of the LDL pathway is the feedback suppression of receptor activity by sterols². When parental CHO and LET-1 cells were incubated with regulatory sterols (cholesterol, the potent analogue 25-hydroxycholesterol²⁰, or esterified cholesterol delivered by LDL^{2,21}), receptor activity was suppressed to essentially the same extent (Table 1, expt *b*). Receptor activity in LET-2 cells was similarly suppressed by LDL (data not shown). The sterol-mediated suppression of LDL receptor activity in human fibroblasts is due to a decrease in the number of LDL receptors¹⁹. Similarly, the suppression of LDL receptor activity in LET-1 cells was associated with a reduction in the amount of immunoprecipitable LDL receptor protein (Fig. 3*b*, compare lane 7 with lane 5). Thus, the expression of the human LDL receptor gene in transfected hamster cells was subject to the same sterol-mediated regulatory control as that observed in normal human² and hamster¹³ cells.

The present studies show that selection in MeLoCo medium can be used to isolate receptor-positive transfectants from a population of receptor-negative (mutant) cells. Using the method of Shih and Weinberg²², it should be possible to clone the LDL receptor gene and combine the techniques of *in vitro* gene manipulation (for example, see ref. 23) and MeLoCo selection to explore the mechanism by which the expression of LDL receptors is regulated. Also, it may be possible to exploit previously described selection methods^{5,6} to isolate mutant LET cells having defects in human LDL receptors and compare these mutant LDL receptors with the naturally occurring mutant LDL receptors from familial hypercholesterolaemia patients¹⁸.

Chemical modification of LDL can be used to re-target the delivery of cholesterol through other surface receptors^{24,25,37}; therefore, it may be possible to use MeLoCo selection to express a wide variety of exogenous receptors (including tissue-specific receptors) in CHO cells and eventually to clone the genes for these receptors. This approach complements a previously described two-step procedure in which cells co-transfected with genomic DNA and a selectable gene were isolated and screened by flow cytometry for the expression of surface antigens (for

example, the transferrin receptor²⁶ and histocompatibility antigens²⁷). In contrast, MeLoCo medium directly selects for receptor function without immunochemical reagents. MeLoCo selection may prove useful for cloning the genes, other than those of receptors, which have been defined by somatic cell mutations to be required for the expression of LDL receptors (ref. 13 and D. Kingsley, M. Segal and M.K., in preparation).

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Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells

W. J. King & G. L. Greene*

Ben May Laboratory for Cancer Research, University of Chicago, 5841 South Maryland, Chicago, Illinois 60637, USA

Although it is widely accepted that specific intracellular receptor proteins are involved in the oestrogenic regulation of gene expression and growth in reproductive tissues, the precise nature of the regulation is poorly understood. Among the unresolved issues are the distribution and dynamics of the oestrogen receptor protein (oestrophilin) in target tissues in the presence and absence of oestrogens and antioestrogens. The use of radio-labelled and unlabelled receptor ligands to detect and measure oestrogen receptors in tissues has been complicated by the presence of other intracellular steroid-binding proteins¹ and by the low concentration of receptors in responsive tissues. We report here the development of an immunocytochemical procedure that is suitable for localizing oestrophilin directly in frozen tissue sections or cells from human and several non-human sources. When monoclonal antibodies to oestrophilin were used to detect receptor in various oestrogen-sensitive tissues, specific staining was confined to the nucleus of all stained cells, suggesting that both cytosol and nuclear forms of the receptor protein may reside in the nuclear compartment.

Five monoclonal rat antibodies (D547Sp γ , D58P3 μ , D75P3 γ , H222Sp γ , and H226Sp γ)^{2–5}, which have been shown to be oestrogen receptor-specific by several criteria, were used individually to localize oestrophilin by an indirect immunoperoxidase technique⁶ in frozen, fixed sections of human breast tumours (Fig. 1), human uterus, rabbit uterus (Fig. 2), and in other mammalian reproductive tissues, as well as in fixed MCF-7 cell cultures (Fig. 3). The nuclear staining observed in each case fulfilled all of the accepted criteria for specificity^{7,8}, including its dependence on the presence (compare Figs 1b, 2b and 3b with 1a, 2a and 3a) and concentration of monoclonal antioestrophilin and the susceptibility of such staining to competitive inhibition by highly purified oestrophilin (Figs 1e, 2e). Specific nuclear staining was either absent or limited to a few cells in receptor-poor breast cancers (Fig. 4); nontarget tissues such as colon epithelium were always negative. Specific staining for oestrophilin in MCF-7 cells, like that in many human breast tumours, was heterogeneous and also confined to the nucleus

(Fig. 3). Staining heterogeneity may reflect the polyclonal origins of these specimens⁹, or it may be related to the physiological status (for example, cell cycle) of the cell populations. Little or no specific cytoplasmic staining for oestrophilin has been observed in any of the tissues or tumour cells examined thus far, including those which have been deprived of exogenous oestrogens for extended times due to postmenopausal amenorrhea or experimental intervention.

For basic studies of receptor dynamics in oestrogen-responsive tissues and cells, the immunoperoxidase technique must be applicable to animal models and cultured tumour cells. Two monoclonal rat antibodies, H222Sp γ and H226p γ , which cross-react with all tested mammalian and avian oestrophilins¹⁰, were used to test the method on oestrogen target tissues from the rabbit. A pattern of predominantly nuclear localization identical to that found in human tissues was observed in sections of rabbit uterus (Figs 2a, 2d), oviduct, corpus luteum, mammary gland, pituitary and liver, but not in colon epithelium. The specificity of the staining patterns in rabbit tissues was confirmed by application of the same controls used for human breast tumours, as shown for rabbit uterus in Figs 2b, 2c and 2e. Animals used in this study were either sexually immature or experimentally deprived of endogenous oestrogens by ovariectomy or induction of pseudopregnancy¹¹. In each case, 70% to 95% of the unoccupied oestrophilin was found in low-salt cytosols, as determined by steroid-binding assays (see legend to Fig. 2).

The nuclear localization of immunoperoxidase staining for oestrogen receptor is surprising as the generally accepted hypothesis is that receptor resides primarily in the cytoplasm of target cells in the absence of steroid^{12–14}. However, early autoradiographic evidence that radioactive steroid incorporated by rat uteri on exposure to ³H-oestradiol at 2 °C *in vitro* is mostly extranuclear¹⁵ has recently been challenged on the basis of similar autoradiographic studies in which binding in the cold appeared to be predominantly nuclear¹⁶. Similar histochemical^{17–19} and immunohistochemical^{20,21} evidence for cytoplasmic receptor sites has been challenged on the basis that the oestrogen derivatives used are bound to lower-affinity intracellular binding sites other than oestrogen receptor¹ or that receptor diffuses out of unfixed frozen sections during localization²². A recent report²³ of cytoplasmic localization of oestrogen receptor with polyclonal antioestrophilin antibodies raised against an impure preparation of human breast tumour oestrophilin lacks supporting evidence of complete antibody specificity for oestrophilin.

It is unlikely that the absence of cytoplasmic staining in the current study is due to the duration of fixation, solubilization of the receptor protein, or to artifactual translocation of receptor during fixation or processing of tissues or cells. The uniformity of our results under a variety of conditions, including treatment of tissues and intact cells with both precipitating and crosslinking fixatives before and after sectioning or freezing, and at temperatures ranging from 4 to 37 °C, suggests that receptor is being permanently immobilized without redistribution. Extending fixation times beyond 1–2 h results only in a progressive loss of nuclear staining without any increase in cytoplasmic staining above background levels. In addition, cytosolic oestrophilin covalently labelled with ³H-tamoxifen aziridine, an antioestrogen derivative²⁴, precipitates quantitatively in 35% aqueous ethanol and remains insoluble when exposed to PBS (unpublished observation). Receptor-containing tissue sections treated with the same fixative show only nuclear staining.

These immunocytochemical results indicate that oestrogen receptor may reside primarily in target cell nuclei of oestrogen-sensitive tissues and tumours both in the presence and absence of steroid, analogous to what has been reported for the vitamin D and thyroid hormone receptors^{25–29}. The oestrophilin recovered in the cytosol fraction of a homogenate would then represent receptor that is loosely associated with the nucleus and it would follow that the binding of oestradiol to its receptor leads to a tighter association, a phenomenon previously interpreted as indicating translocation of the receptor from the

* To whom correspondence should be addressed.

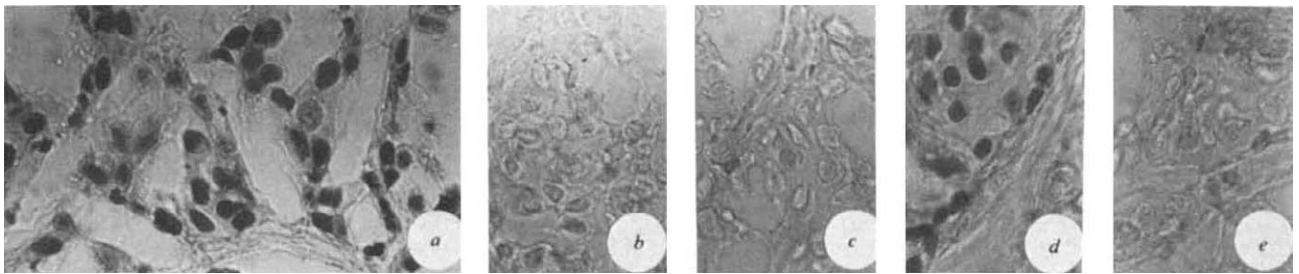


Fig. 1 Immunocytochemical localization of oestrogen receptor in a breast tumour taken from a 67-year-old postmenopausal woman, and which contained 1,546 fmol of cytosolic oestrogen receptor per gram wet weight, as measured by sucrose density gradient analysis³. Frozen sections (8 μ m) were thaw-mounted on uncoated glass slides and fixed with picric acid-paraformaldehyde³⁰ for 10 min at 23 °C. After fixation, sections were rinsed with phosphate-buffered saline (PBS) and treated with a 10% (w/v) solution of ovalbumin in PBS to minimize the nonspecific binding of reagents in subsequent steps. Tissue sections were then incubated successively with one or more monoclonal antibodies, goat antibody to rat IgG, and rat peroxidase-antiperoxidase complex (PAP) for 30 min each at ambient temperature in a humidified chamber. Each incubation was followed by a 5-min wash with PBS. Specifically bound antibodies were visualized by immersing the sections for 10–30 min in a solution of diaminobenzidine (1.7 mM) and hydrogen peroxide (0.06%) in PBS. *a–e*, sections were treated identically, except for the first incubation which consisted of either (*a*) 2 μ g ml⁻¹ (13.4 pmol ml⁻¹) of H226Spy antibody in PBS; (*b*) 2 μ g ml⁻¹ of purified normal rat IgG in PBS; (*c*) 2 μ g ml⁻¹ of H226Spy in MCF-cytosol (20 pmol receptor per ml); (*d*) 2 μ g ml⁻¹ of H226Spy in receptor-depleted (by selective adsorption to estradiol-Sepharose⁴) MCF-7 cytosol (2 pmol receptor per ml); or (*e*) 2 μ g ml⁻¹ of H226Spy in receptor-depleted MCF-7 cytosol supplemented with 20 pmol ml⁻¹ of highly purified (~90% pure) oestrophilin^{10,31}. Original magnification, $\times 400$. The specificities of the antibodies used in this study were tested extensively by Western blot, sucrose gradient^{2–4}, double antibody precipitation² and indirect enzyme immunoassay (EIA) techniques. Regardless of the technique employed, each of the antibodies appears to be completely specific for oestrogen receptor. When ³⁵S-labelled H222Spy and H226Spy IgGs were used to detect immunoreactive proteins on nitrocellulose blots of SDS gels after electrophoresis of low and high salt extract of MCF-7 cells, both antibodies recognized a single protein band (molecular weight, 65,000), which corresponded to highly purified receptor. The same band was obtained when crude oestrophilin was isolated by immunoabsorption to a column of D547Spy-Sepharose and eluted with SDS.

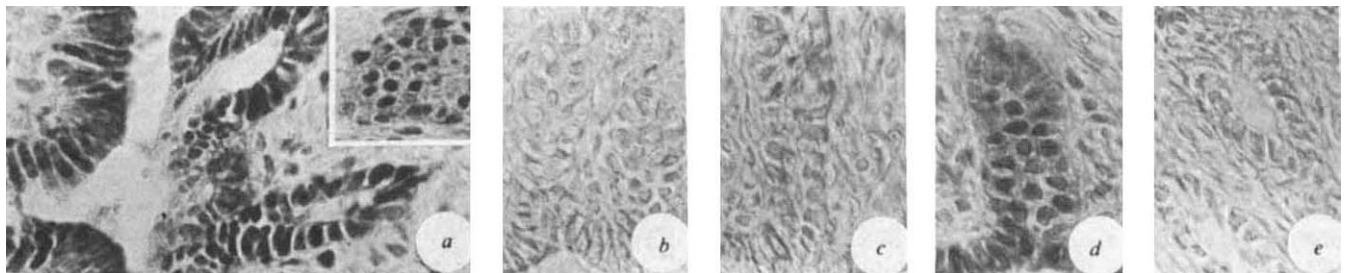


Fig. 2 All procedures were the same as Fig. 1, except that sections were prepared from a uterus taken from a rabbit ovariectomized 8 days prior to killing. Uteri from similarly treated rabbits were found to contain 3.15 ± 0.85 pmol cytosolic receptor per mg of DNA ($n = 3$) and 0.16 ± 0.01 pmol of high-salt (0.4 M KCl) extractable receptor per mg of DNA, as measured by exchange assay with ³H-oestradiol³². The insert in the upper right corner of Fig. 2a represents rabbit myometrium.

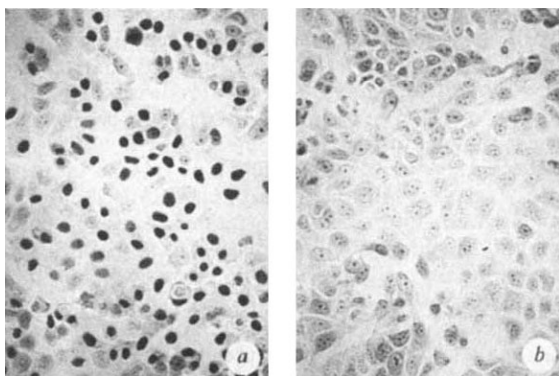


Fig. 3 Localization of oestrogen receptor in MCF-7 human breast cancer cells. Cells were grown on chambered glass slides, fixed for 10 min with picric acid-paraformaldehyde³⁰ and oestrogen receptor was localized as described in Fig. 1 using 10 μ g ml⁻¹ of H226Spy antibody in PBS (*a*). Cells shown in (*b*) were treated identically except that 10 μ g ml⁻¹ of normal rat IgG was substituted for the monoclonal antibody.

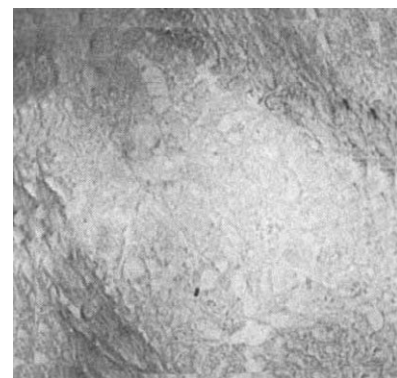


Fig. 4 Absence of nuclear staining in a human receptor negative (0 fmol g⁻¹ tissue) breast tumour from a postmenopausal patient. Staining conditions were as described in Fig. 1, using 10 μ g ml⁻¹ of H226Spy antibody in PBS. Original magnification, $\times 400$.

cytoplasm to the nucleus¹². In support of our hypothesis, we have seen little or no increase in nuclear staining intensity in MCF-7 cells and immature or ovariectomized rabbit uteri following short-term treatment of cells or animals with oestradiol. Our observations are clearly inconsistent with the concept of steroid-induced receptor translocation, suggesting that the classical 'two-step' model¹² for oestrogen action requires re-interpretation.

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Nuclear localization of unoccupied oestrogen receptors

Wade V. Welshons, Mara E. Lieberman & Jack Gorski

Departments of Biochemistry, Human Oncology and Animal Science, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA

According to the current model of steroid hormone action oestrogen is thought to bind to its receptor in the cytoplasm of target cells^{1,2} and the oestrogen-receptor complex is then translocated into the nucleus²⁻⁴. This model is based on evidence obtained in homogenized cell preparations in which free receptor is associated with the cytosol, whereas steroid-bound receptor is associated with the nuclear fraction. Some data suggest, however, that the unoccupied receptor may reside in the nucleus, and that cytosolic localization represents an extraction artefact. We have now reinvestigated the subcellular distribution of unoccupied oestrogen receptor using cytochalasin B-induced enucleation to obtain cytoplasm and nucleoplasm fractions from receptor-containing GH₃ cells derived from rat pituitary tumours. We found that cytoplasm prepared from GH₃ cells contain little oestrogen-binding activity and that most of the unoccupied oestrogen receptors are associated with the nuclear fraction. We therefore suggest that the standard model is in error and that the unoccupied receptor is nuclear in the intact cell.

If unoccupied oestrogen receptor is cytoplasmic, the receptor concentration in the cytoplasm should be greater than the concentration in whole cells. In fact, when cells were enucleated (Fig. 1) the concentration of receptor relative to protein was greatly reduced in cytoplasm and increased in the fraction containing cells and nucleoplasm compared with intact cells (Fig. 2). In contrast, receptor relative to DNA was similar in all cell preparations (Fig. 2), indicating that the receptor is associated with nuclear rather than cytoplasmic elements. In an experiment using cytoplasm that contained particularly few contaminating whole cells (0.25%), the concentration of receptor relative to protein was less than 10% of that found in intact cells (Table 1). In four enucleation experiments the recovery of protein, DNA or oestrogen receptor was 93, 97 or 104%, respectively. The essentially complete recovery indicates that receptor was not selectively lost from cytoplasm.

The cell+nucleoplasm fraction was further fractionated by centrifugation to obtain cells from which a larger proportion of the cytoplasm had been removed. Receptor more than doubled relative protein (Fig. 3, fraction C), whereas the receptor: DNA ratio was constant.

The cytoplasm obtained following enucleation are approximately one-fifth the size of whole cells, according to protein content (Table 1) and 75% or more of the cells appear to form cytoplasm (Fig. 1). The cytoplasm exclude trypan blue (98%), incorporate ³H-leucine into protein generally and prolactin specifically (data not shown). Specific oestrogen binding by whole cells (or whole cytoplasm) was saturable with K_d of 3.4×10^{-10} M by Scatchard analysis, and there were usually 20,000-30,000 oestrogen-binding sites per cell.

Incubation of whole cells in enucleation medium (Percoll, cytochalasin B, dimethyl sulphoxide (DMSO)) did not significantly change the number of receptors per cell or the distribution of receptor between the cytosol and the crude nuclear pellet in homogenized cells (data not shown). This confirms earlier studies with intact uteri in which cytochalasin had no effect on the uptake of oestradiol, the binding, or the distribution of filled receptors in homogenized tissue⁵.

Our finding that the oestrogen receptor is predominantly nuclear, whether or not it is bound to the steroid, contradicts a large body of evidence which has been interpreted to indicate that the free oestrogen receptor is a cytoplasmic protein. There are, however, several earlier reports in the literature which are compatible with our findings. These include autoradiographic localization of most of hormone binding to the nucleus in 'non-translocation' conditions⁶, appearance of 4S 'cytoplasmic' receptor in nuclei of some tissue before 5S 'nuclear' forms which appear after binding of the hormone⁷, and evidence that some oestrogens produce full physiological effects even though the receptor appears entirely in cytosol extracts of homogenized cells^{8,9}. The unoccupied vitamin D receptor and thyroid hormone receptor have been reported to be nuclear in homogenized cells^{10,11}, and cell lines have been described in which unoccupied oestrogen receptor remains nuclear in homogenized cells¹² until nuclei are washed and extracted more rigorously¹³.

In contrast to earlier immunohistochemical studies which showed cytoplasmic localization of receptor¹⁴⁻¹⁶, a recent immunocytochemical study indicates that antibodies to the oestrogen receptor interact only with nuclei of human endometrium or human mammary tumour cells, whether or not oestrogen is present¹⁷.

In the present study we used GH₃ cells, a clonal strain derived from a rat pituitary tumour^{18,19} that contain oestrogen receptors²⁰ localized in the cytosol after the usual cell disruption procedures^{20,21}. GH₃ cells, including the specific strain used here, respond to oestrogen by increased prolactin synthesis^{22,23}. The concentration of oestrogen receptor in cytoplasm was only 5-10% of that observed in intact cells. This was not due to degradation of receptor during enucleation, because all of the binding activity was recovered in the fraction enriched in nuclei. It is unlikely that the cytoplasm were formed from a small, atypical subpopulation of cells that were deficient in receptor,

Fig. 1 Feulgen-stained whole cells (a) and cytoplasts (b) or cells+nucleoplasts (c) after enucleation. Enucleation of GH₃ cells yielded cytoplasts with diameters up to approximately 50% that of whole cells, and nucleoplasts plus cells with varying amounts of cytoplasm. Scale bar, 10 μ m.

Methods: GH₃ cells were grown in Dulbecco's minimum essential medium (DMEM, Gibco) containing HEPES 25 mM, penicillin (Sigma) 100 U ml⁻¹, Fungizone (Gibco) 0.5 μ g ml⁻¹ and 17.5% horse-fetal calf serum (Gibco) 6:1. Charcoal-stripped serum was used throughout, or for the last medium change, with similar results. Cells were removed from the tissue culture dish by incubating in 1 mM EDTA in calcium-magnesium-free Hanks' balanced salt solution +25 mM HEPES (CMFH) for 10 min at 37 °C. Cells were enucleated by the cytochalasin B-equilibrium centrifugation method of Bossart *et al.*²⁴, with modifications. For preparation of cytoplasts that were contaminated with few nucleated cells, a narrow density range of cells was selected before enucleation. This was accomplished by applying cells (20–40 $\times 10^6$ per 13-ml tube) to a step-gradient of Percoll (Pharmacia) in CMFH, with steps of 1.04, 1.05, 1.058, 1.063 and 1.07 g ml⁻¹. After a 20-min spin at 400g in a swinging bucket centrifuge, the predominant band was collected, usually at 1.058–1.063 g ml⁻¹. The cells were diluted 5 $\times$ in CMFH, pelleted (125g, 10 min) and resuspended in Percoll of 1.075 g ml⁻¹ (for cells from 1.058–1.063 g ml⁻¹), containing cytochalasin B (Aldrich) at 20 μ g ml⁻¹ and solvent DMSO 20 μ l ml⁻¹, 5–10 $\times 10^6$ cells per ml. The cells were incubated at 37 °C for 45 min, then layered onto more of the Percoll plus cytochalasin B (10–20 μ g ml⁻¹), 37 °C, in a clear ultracentrifuge tube, and then spun at 64,000g for 45 min at 37 °C in a Beckman SW41 swinging bucket rotor. At 1 $\times 10^7$ cells per gradient (12 ml total), cytoplasts could be recovered with minimal contamination by whole cells (<1%), but for higher yields, up to 40 $\times 10^6$ cells were applied, which resulted in cytoplast preparations that contained up to 5% whole cells. After centrifugation, the cytoplasts were collected from a middle quarter of the tube. The very fine uppermost band of dead cells was avoided. The lowest layer of cells+nucleoplasts was also collected, and after a 5 $\times$ dilution with CMFH, fractions were pelleted (125g 10 min for cells, 275g 10 min for cytoplasts). Subsequent centrifugation for cells was at 80g, 10 min. Generally, 10 $\times 10^6$ cells applied per gradient yielded 7.5 $\times 10^6$ cytoplasts. For Feulgen staining, cells were fixed in 10% formalin in CMFH overnight, dried and ethanol-fixed onto slides, and stained by the Feulgen procedure. Cells were counterstained for 30 s in 0.5% Wool green (CI 44090, Aldrich) in 0.5% acetic acid, rinsed briefly in water, air dried, and mounted in balsam.

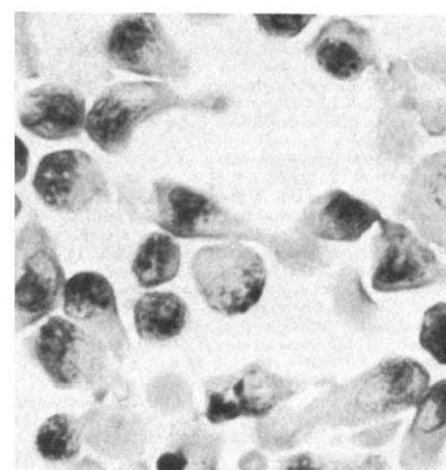
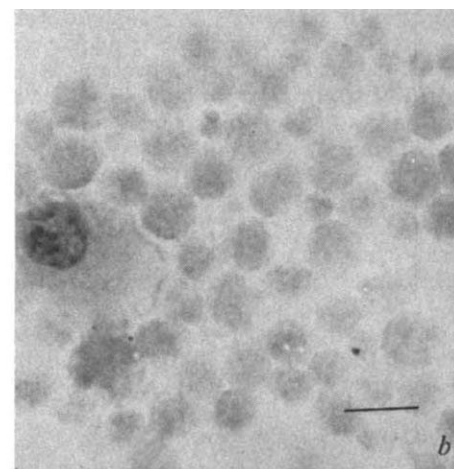
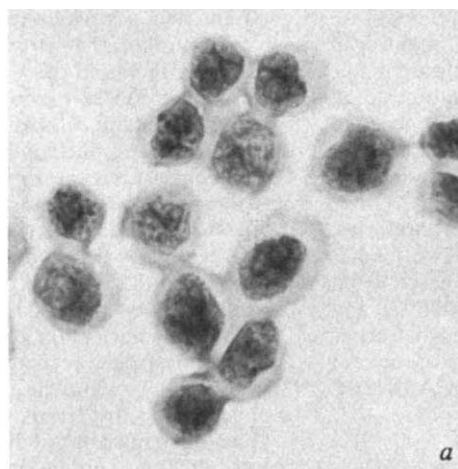


Fig. 2 Intracellular distribution of oestrogen receptor (ER). GH₃ cells were enucleated as described in the text. Oestrogen receptor, protein and DNA were measured in untreated density-selected whole cells (WC), in the cell + nucleoplast fraction (C+N), and in the cytoplast fraction (Cyt). In intact cells or cytoplasts, oestrogen receptor was measured as specific uptake of ³H-oestradiol (151 Ci mmol⁻¹, NEN) 2 nM in culture medium + DNase (Worthington DPFF 50 μ g ml⁻¹) after 30 min at 37 °C, with or without 200 nM nonradioactive oestradiol. Protein was measured with Coomassie blue staining²⁵ using the Bio-Rad microassay with bovine γ -globulin (Sigma) as the standard. DNA was measured using diphenylamine²⁶ or the fluorescent dye Hoechst 33258²⁷. The figure shows the results of three separate experiments (error bar is the standard error) in which cytoplasts contained 4%, 2.5%, and 0.25% contaminating whole cells.

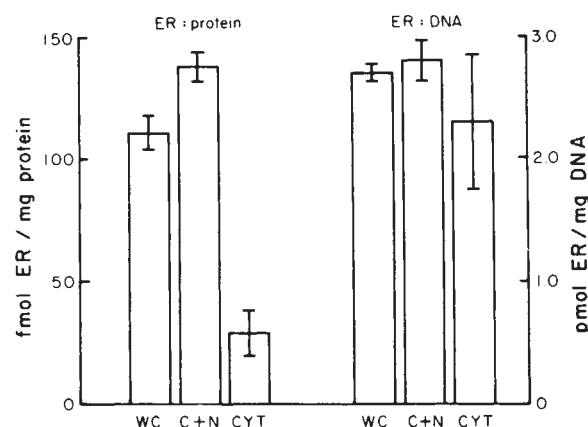


Table 1 Oestrogen receptor in enucleated cells

	Oestrogen receptor		Protein per cell (pg)	DNA per mg protein (μ g)	Oestrogen receptors per cell (molecules)
	per mg protein (fmol)	per mg DNA (pmol)			
Whole cells	113	2.7	363	42	25,000
Cells+nucleoplasts	146	2.9	336	50	30,000
Cytoplasts	10	1.4	69	7.5	2,100*

One of the experiments of Fig. 3. Cytoplasts contained only 0.25% contaminating whole cells, by cell count with haemocytometer.

* As cytoplasts are approximately one-fifth the size of whole cells, by protein content, cytoplast receptors: cell equivalent was calculated as 5 $\times$ the actual number of receptors per cytoplast.

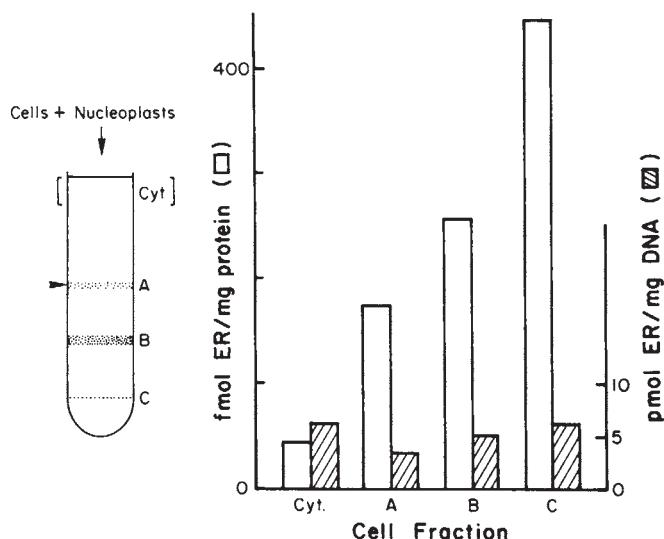


Fig. 3 After enucleation, the cytoplasts were saved separately while the cell+nucleoplast layer was further fractionated on a density step gradient, with Percoll at 1.04, 1.05, 1.07, 1.08 and 1.10 g ml⁻¹. After 20 min at 400g, cells were collected at interfaces between 1.05 to 1.07 g ml⁻¹ (A), 1.07 to 1.08 g ml⁻¹ (B), and 1.08 to 1.10 g ml⁻¹ (C). The arrowhead at A indicates where the density-selected whole cells (1.058–1.063 g ml⁻¹) would have been found before enucleation, and the position that the cytoplasts would have occupied is indicated in brackets. Oestrogen receptor, DNA and protein were measured in each fraction. Of the cells+nucleoplasts that were recovered after fractionation by density, 15% were recovered at A (which includes the pre-enucleation density), 76% were recovered at B, and 9% were recovered at C.

since the majority of the cells appeared to contribute a cytoplast (Fig. 1). Furthermore, at least 85% of the cells increase in density after enucleation (Fig. 3 legend), which is consistent with this hypothesis.

Thus, the unfilled oestrogen receptor appears to be largely nuclear in the intact GH₃ cell. We propose that in the absence of steroid, the loose association of the receptor with the nuclear elements causes the receptor to partition into the cytosol when the cell is disrupted, and thus the cytosolic receptor represents an extraction artefact. This phenomenon may be limited to the GH₃ cell, but the similarity of the different steroid receptor systems suggests that these findings may be more broadly applicable. From our data and the literature cited above, we propose that in the intact cell, there is no nuclear translocation of receptor as part of the steroid response, but rather an increase in receptor affinity for nuclear elements.

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Novel κ light-chain gene rearrangements in mouse λ light chain-producing B lymphocytes

Jeannine Durdik, Mark W. Moore & Erik Selsing

Rosenstiel Basic Medical Sciences Research Center and Department of Biology, Brandeis University, Waltham, Massachusetts 02254, USA

The genes that encode the immunoglobulin proteins made by B lymphocytes are made up of segments that are separately encoded in the germ-line genome and brought together by recombination during B-cell ontogeny¹. There are two types of immunoglobulin light chain, κ and λ , but only a single type is expressed in individual B cells. It is thought that κ gene recombination precedes λ gene recombination during B-cell ontogeny^{2,3}. We describe here unusual recombinations that have occurred in two λ -producing B-cell lines and suggest that they are involved in the developmental switch from κ to λ gene expression in maturing B cells. These recombinations involve the J_{κ} - C_{κ} introns of V-J joined but nonfunctional κ genes and a sequence that in the germ line occurs downstream of the C_{κ} exon (called RS, for recombining sequence).

We first detected κ DNA segments having unusual hybridization characteristics in Southern blot analyses⁴ of two λ -producing cell lines, CH2 and MOPC315. In these cells, we found segments of DNA that hybridized with J_{κ} probes and intron probes but not with C_{κ} probes or probes representing sequences 5' of the J_{κ} region (Fig. 1). These results indicated that J_{κ} -containing DNA segments in CH2 and MOPC315 had apparently undergone two recombinational events leading to the removal or replacement of DNA on both the 5' and 3' sides of the germ-line J_{κ} region. These recombinational events have resulted in J_{κ} regions having no associated C_{κ} exons in CH2 and MOPC315 cells. Indeed, both CH2 and MOPC315 have no C_{κ} exons within their genomes (Fig. 1); this is often the case in λ -producing cell lines^{5,6}.

We isolated and sequenced portions of the unusual J_{κ} -containing DNA segments from CH2 and MOPC315 cells to determine their structures (Fig. 2). Our data show that the two recombinational events generating the unusual DNAs in CH2 and MOPC315 have occurred within the J_{κ} locus and within the J_{κ} - C_{κ} intron. The J_{κ} -associated recombinations in CH2 and MOPC315 cells represent V-J joining events similar to those observed in all immunoglobulin-producing B cells (sequence data not shown). Alignment of the DNA sequences, using the invariant amino acid positions of mouse V_{κ} proteins⁷ and the DNA sequences of the germ-line J_{κ} locus⁸, shows that these V-J joining events are nonfunctional due to misalignments and frameshifts similar to others observed in several recombined antibody genes^{9–11}. On the other hand, the J_{κ} - C_{κ} intron-associated recombinations that we find in the M315-rs1 and CH2-rs3 clones (Fig. 2) do not resemble any previously reported immunoglobulin gene recombinations. It is striking that our restriction map and sequence data indicate that the recombined

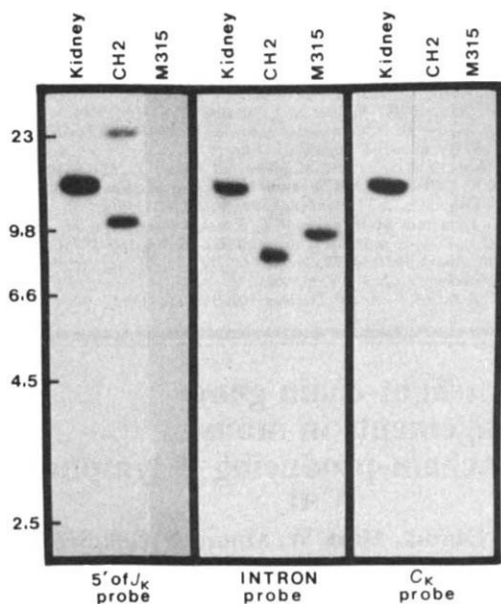


Fig. 1 Recombination of κ -gene DNA in CH2 and MOPC315 myelomas. Southern blots of $\sim 30 \mu\text{g}$ of the indicated genomic DNAs, digested with *Bam*HI, were hybridized with the probes indicated. The probes are described in Fig. 2. Sizes of marker λ phage *Hind*III restriction fragments are indicated. RS DNA recombinations are detected as DNA fragments that hybridize with intron probes (and with J_κ probes, not shown) but not with C_κ probes or probes 5' of J_κ . The DNA fragments in CH2 cells that are detected by 5' of J_κ probes but not by intron or C_κ probes have been reported previously²¹. These DNA fragments seem to be related to κ gene V-J joining and will be described in detail elsewhere (E.S., J. Voss and U. Storb, in preparation).

RS DNA segments in CH2 and MOPC315 cells are identical. Our sequences also localize the joining sites for the intron-associated RS DNA recombinational events in rs3 and rs1 to within three nucleotides of each other (Fig. 2).

Because the recombined RS DNA segments found in CH2 and MOPC315 cells seem identical, we prepared unique hybridization probes for RS sequences from our rs1 and rs3 clones to ascertain the status of RS DNA in other cell lines. Figure 3 shows that RS sequences appear to be present in one copy per haploid genome in normal mouse kidney cells as judged by the intensity of a single germ-line hybridization band. In various murine B-cell tumour lines, however, RS DNA is often recombined (Fig. 3). In some cell lines, only a recombined RS segment is observed, reinforcing the notion that RS DNA is a single-copy DNA segment. In total, we found RS recombinations in four of six κ -producing plasmacytomas tested and also in all five λ -producing cell lines screened (see Fig. 3 legend). Of the 10 recombined RS segments that we observed in B-cell tumours, only the two in CH2 and MOPC315 hybridized with J_κ or intron probes (Fig. 1) indicating that many RS recombinations apparently involve sequences other than the J_κ - C_κ region. No RS recombinations were present in 12 T-cell tumour lines (data not shown). When we screened antibody-producing hybridomas we found that, in 14 κ -producing hybrids, only the recombined RS sequences contributed by the myeloma parent were present; no recombined RS DNA segments from the normal B-cell partner were detected (data not shown). In contrast, out of 10 λ -producing hybridomas, 8 displayed one or more recombined RS sequences apparently coming from the normal B-cell fusion partner (Fig. 3). We discuss the contrasting data from the normal and transformed B cells below; the results from the hybridomas, however, indicate that RS recombination is frequently linked to λ light-chain gene expression.

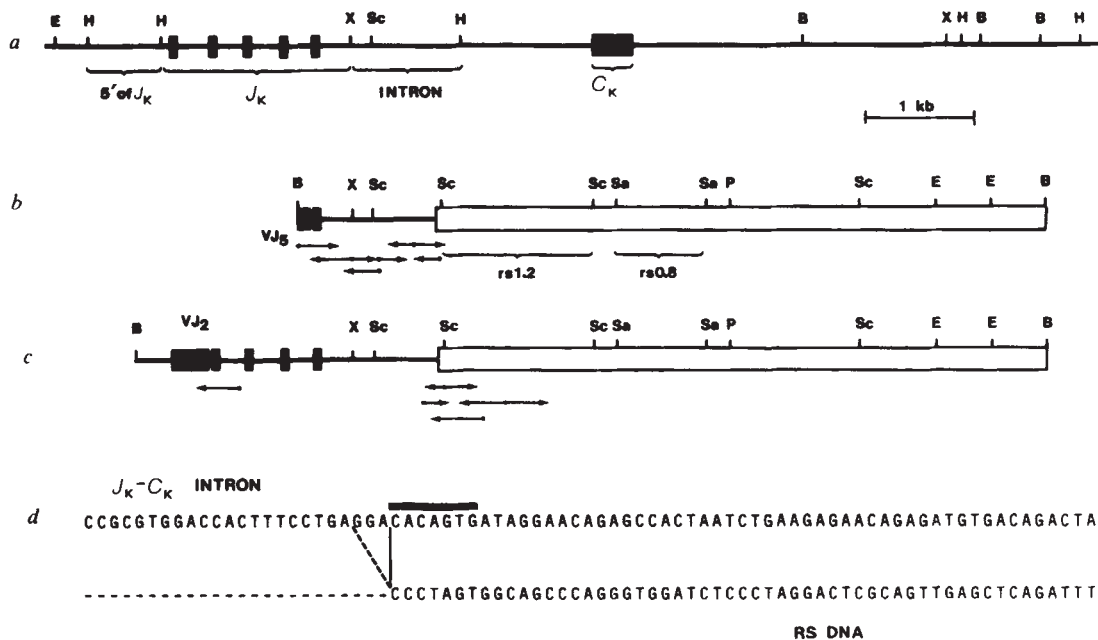


Fig. 2 Restriction enzyme maps of RS DNA clones. Restriction sites within the germ-line J_κ - C_κ locus and hybridization probes that were used to detect RS DNA recombination in MOPC315 and CH2 cells are shown in *a*. *b* and *c* show restriction maps of recombined RS DNAs (CH2-rs3 and M315-rs1) in CH2 and MOPC315 cells, respectively. Segments of rs3 and rs1 that comprise V_κ and J_κ sequences are indicated as lines or solid bars. Portions of rs3 and rs1 that derive from RS DNA are indicated as open bars. The positions of the rs1.2 and rs0.8 probes are also indicated. Chain termination DNA sequence analyses²² of rs3 and rs1 *Sau*3A, *Hae*III or *Bam*HI, *Xba*I restriction fragments using M13 sequencing vectors²³ are indicated by arrows in *b* and *c*. All maps are drawn to scale and aligned at the *Xba*I site within the J_κ - C_κ intron. Restriction enzymes are: *Eco*RI (E), *Hind*III (H), *Xba*I (X), *Sac*I (Sc), *Sau*3A (Sa) and *Bam*HI (B). No *Hind*III sites are present in CH2-rs3 or M315-rs1. Not all *Sau*3A sites have been mapped in the rs3 and rs1 clones. *d* Shows nucleotide sequences surrounding the RS DNA recombination sites in rs3 and rs1. The diagonal dashed line represents RS recombination in CH2; the vertical solid line is RS recombination in MOPC315. An immunoglobulin recognition site heptamer, localized near CH2 and MOPC315 RS DNA recombination sites within the germ-line J_κ - C_κ intron region^{8,14}, is indicated by a heavy bar. Our J_κ - C_κ intron sequence differs at a few positions from that reported by Max *et al.*⁸; we find the same sequences in the rs3 and rs1 clones and presume that the previous sequence is in error. The horizontal dashes represent sequences 5' of unrecombined RS DNA; these have not yet been determined.

Sequences near the sites of RS DNA recombination in CH2 and MOPC315 demonstrate an intriguing feature. In the J_{κ} - C_{κ} intron segment of DNA which is replaced by RS sequences, an immunoglobulin recognition site heptamer is present (Fig. 2). This heptamer represents one portion of the bipartite conserved recognition sequences that are found next to immunoglobulin V, D and J segments and that have been proposed to be binding sites for proteins involved in V-J and V-D-J antibody gene recombination^{12,13}. Thus, the RS DNA recombinations in CH2 and MOPC315 bear a remarkable resemblance to the immunoglobulin gene recombinations that occur in normal differentiating B cells. This suggests that some enzymatic activities involved in normal B-cell antibody gene recombination could also have a role in RS DNA recombination. The recognition site heptamer located near RS DNA recombinations in CH2 and MOPC315 has previously been shown to be involved in an aberrant V_{κ} - C_{κ} recombination event in the myeloma, MPC11 (ref. 14).

Using Chinese hamster-mouse fusion cell lines, we have localized RS sequences to chromosome 6 in the mouse (J.D., D. Pravtcheva, F. Ruddle and E.S., unpublished). The structures of CH2-rs3 and M315-rs1 (Fig. 2) suggest that RS DNA may lie downstream of the C_{κ} exon on chromosome 6 and that the V_{κ} - J_{κ} -RS recombinations in CH2 and MOPC315 cells have resulted in deletions of C_{κ} (Fig. 1). Using various probes we can detect no homology between our RS DNA clones and a germ-line J_{κ} - C_{κ} clone (Charon 4A-Sp101) described previously¹⁵. Thus, RS sequences seem to be localized more than 10 kilobases (kb) downstream of the C_{κ} exon.

It is unknown which sequences recombine with RS in those cell lines where RS recombination does not involve the J_{κ} - C_{κ} intron. However, because the RS recombinations in CH2 and MOPC315 cells involve a DNA sequence that resembles an immunoglobulin gene recognition site, it is possible that some RS DNA recombinations represent joining with V_{κ} genes. If RS sequences are downstream of the C_{κ} exon then such putative V_{κ} -RS recombinations would presumably also result in the deletion of C_{κ} (and J_{κ}) regions. These observations suggest that we have detected, for the first time, the molecular events responsible for the deletions of C_{κ} and J_{κ} exons that have frequently been observed in mouse λ -producing myelomas^{5,6}. It will be interesting to determine whether sequences similar to RS are found in human cells because human λ -producing B-cell lymphomas also commonly have lost the J_{κ} - C_{κ} region².

Do the RS DNA recombinations that we have found have functional significance during B-cell ontogeny? The apparent lack of RS recombinations in two λ -producing hybridomas (Fig. 3), although possibly due to chromosomal loss during the cell fusion and selection process, does indicate that recombined RS segments are not essential for continued λ -chain production in cells that have functional, V-J joined λ genes. It could be that RS recombinations specifically act as mechanisms to remove C_{κ} regions from the genomes of B lymphocytes. It has been suggested that C_{κ} deletion might serve to remove nonfunctional V-J joined κ genes from the genomes of B lymphocytes and thus eliminate aberrant transcripts and prevent the synthesis of incomplete light chains¹⁶. Alternatively, RS recombination might have a positive role in B-cell differentiation. The structures of CH2-rs3 and M315-rs1 place RS DNA directly downstream from potentially active immunoglobulin gene promoters associated with recombined V_{κ} regions^{17,18}. The putative V_{κ} -RS recombinations mentioned above would also exhibit structures with RS downstream from V_{κ} gene promoters. It is possible that RS DNA contains a gene that could be transcriptionally activated by recombination. Our finding of RS recombinations only in λ -producing hybridomas and not in κ -producing hybridomas suggests that such a gene might be involved in a switch from κ recombination to λ recombination in maturing B lymphocytes. In this model, C_{κ} exon deletion would be a side effect of RS recombination events.

We have sequenced ~900 base pairs of RS DNA adjacent to the recombination site in M315-rs1 (see Fig. 2). Within this sequence there are several short potential protein-encoding

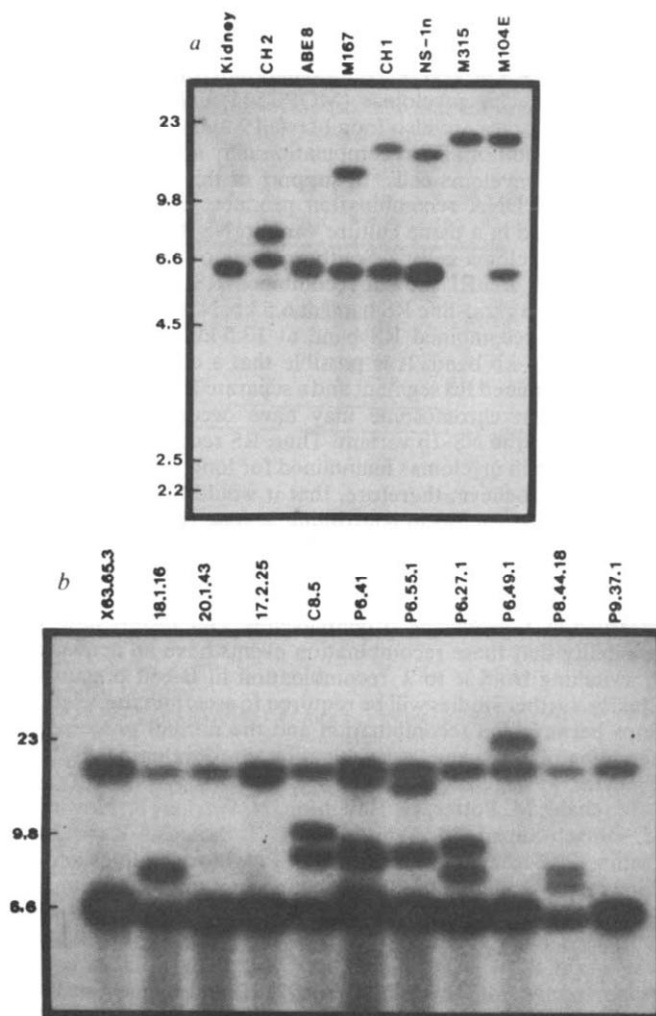


Fig. 3 RS recombination in B-cell lines. *Eco*RI-digested genomic DNAs were probed with the rs0.8 fragment (see Fig. 2). In *a*, recombined RS segments are seen in the κ -producing myelomas, MOPC167 (Litton) and NS-1n²⁰; in the λ -producing myelomas, MOPC315 and MOPC104E (Litton); and in the λ -producing B lymphomas, CH1 and CH2 (ref. 24). In the Abelson murine leukaemia virus-transformed cell line, ABE8 (ref. 25), only a germ-line RS segment is evident. In other experiments we have detected recombined RS segments in the κ -producing myelomas MOPC511, MOPC321 and MOPC21 (Litton) and in the λ -producing myeloma, S178 (Litton). Only germ-line RS segments were found in the κ -producing myelomas, MOPC41 and J606 (Litton). In the 12 T-cell lines, SL1-SL9 (ref. 26), BALENTL-5 (Litton), EL-4 and YAC-1 (Salk), we found only germ-line RS segments. In *b*, recombined RS segments are seen in 8 of 10 anti-NP λ -producing hybridomas (ref. 27 and M. Borsch-Supan and T. Imanishi-Kari, unpublished) compared with the myeloma fusion partner (X63.65.3) used in the cell fusions. In the hybridoma, 17.2.25, a recombined RS band at ~13 kb can be resolved from the X63.65.3 parental RS band in the original autoradiogram. An analogous experiment with 14 κ -producing hybridomas showed no recombined RS segments other than those from the myeloma fusion partner. κ Hybridomas included the six anti-PC hybridomas, 101.3C2.1, 101.3G8.4, 101.6G6.2, 100.6F9.1, 137.5G6 (ref. 28) and HP16 (ref. 29) and eight anti-(T,G,A)-L hybridomas (J. Press, unpublished).

open reading frames (40–80 amino acids long) but no clearly relevant RNA transcription or processing signals. We have not yet found any RNA transcription products from recombined RS segments using Northern blots of MOPC315 poly(A)⁺ RNA hybridized with the rs0.8 and rs1.2 probes shown in Fig. 2 (J.D., S. Ogden, U. Storb and E.S., unpublished). To rule out a potential role of RS transcription in λ gene recombination, however, it will be necessary to probe cells actually undergoing immunoglobulin gene recombination.

The RS DNA recombinations that we observe in four κ -producing myelomas do not seem to be consistent with a regulatory role for RS in λ gene recombination. However, in two of these κ -producing myelomas (MOPC511 and MOPC21) recombined λ genes are also found (ref. 19 and our unpublished results). In addition, RS recombination may not be completely repressed in myeloma cells. In support of this notion, we find different RS DNA recombination products in the myeloma, MOPC21, and in a tissue culture variant, NS-1n, derived from MOPC21 myeloma cells²⁰. Southern blots of MOPC21 DNA digested with *Eco*RI show a recombined RS band at 12.5 kb and an intense germ-line RS band at 6.5 kb. NS-1n, on the other hand, has a recombined RS band at 13.5 kb together with a germ-line 6.5-kb band. It is possible that a chromosomal loss of one recombined RS segment and a separate RS recombination on a different chromosome may have occurred during the generation of the NS-1n variant. Thus, RS recombinations may not be stable in myelomas maintained for long periods by serial passage. We believe, therefore, that it would be premature to discount a role for RS in controlling λ gene recombination on the basis of two κ -producing tumours.

Apparently, we have discovered molecular recombination events involved in the C_{κ} -deletion phenomenon commonly observed in λ -producing B lymphocytes. Our results raise the possibility that these recombination events have an active role in switching from κ to λ recombination in B-cell precursors. Clearly, further studies will be required to ascertain the relationships between RS recombination and the normal processes of immunoglobulin gene recombination in developing B lymphocytes.

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A variant translocation places the λ immunoglobulin genes 3' to the *c-myc* oncogene in Burkitt's lymphoma

G. F. Hollis, K. F. Mitchell, J. Battey, H. Potter, R. Taub, G. M. Lenoir* & P. Leder

Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA

* International Agency for Research on Cancer, Lyon, France

Most translocations that occur in Burkitt's lymphoma^{1,2} involve movement of part of chromosome 8, containing the *c-myc* gene, from its normal position to the immunoglobulin heavy-chain locus on chromosome 14³⁻⁷. The genes are often joined at their 5' ends in opposite transcriptional directions⁴⁻¹³. However, a significant minority of Burkitt translocations⁸ involve the light-chain loci on chromosome 2 (κ)¹⁴ or 22 (λ)¹⁵. We have characterized one of these from a European-derived cell line (IARC-BL37) that carries an 8;22 translocation. Here the translocation has joined the 5' portion of the λ light-chain locus to the 3' portion of the *c-myc* gene at a position about 7 kilobases from the normal *c-myc* promoters. The translocation is reciprocal and relatively conservative, involving the loss of only 21 base pairs from the site of recombination. This translocation allows us to orient the λ genes with respect to the centromere of chromosome 22 and to predict the orientation of other translocations involving these chromosomal segments. The 3' translocation is accompanied by an increased level of *c-myc* transcripts, especially that derived from a normally under-used *c-myc* promoter.

The Burkitt's lymphoma line BL37 is a European-derived, Epstein-Barr virus (EBV) positive cell line that carries an 8;22 translocation and produces immunoglobulin μ and κ chains. In our hands, this line is the only one for which the previously described correlation between type of translocation and light-chain expression⁸ is not observed, and the first line with the 8;22 translocation to show a detectable rearranged *c-myc* gene. Comparison of *Eco*RI restriction fragments derived from the genomic DNA of the Burkitt cells with those from normal tissue (Fig. 1, lanes 1, 2) showed that both tissues contain a 12.5 kilobase (kb) *Eco*RI fragment corresponding to the germ-line *c-myc* gene. The Burkitt cell contained an additional fragment, 19.5 kb in length, presumably corresponding to the rearranged *c-myc* gene. If this rearranged fragment contains the immunoglobulin λ light-chain genes¹⁶, as suggested by the fact that that translocation involves chromosome 22, we might further expect to find a correspondingly rearranged *Eco*RI fragment of DNA that hybridizes with a probe derived from the λ constant region. The relevant experiment is also shown in Fig. 1 (lanes 3, 4) in which a λ constant region probe detects a single rearranged fragment, also 19.5 kb in length, in addition to the normal bands at 5, 8, 14 and 16 kb. (The 5 kb *Eco*RI fragment seen in the middle lanes corresponds to a processed λ pseudogene unlinked to the λ locus¹⁷.) Note that these λ *Eco*RI fragments maintain their germ-line configuration, as would be expected in a κ -producing B cell^{18,19}.

These data suggest that the *c-myc* and the immunoglobulin λ gene sequences now exist on a 19.5 kb *Eco*RI fragment of DNA. While any of the six λ genes represented in this haplotype could have been involved in this rearrangement, systematic studies (data not shown) with uniquely hybridizing probes derived from the flanking sequences 5' to the first three λ genes suggested that the rearrangement occurred 5' to the λ_1 gene (see Fig. 1). Evidence that the recombination between the chromosomes is a reciprocal event was obtained using a probe from the region 5' to the cross-over point of recombination (Fig. 1). The experiment is shown in the lanes 5 and 6 of Fig. 1

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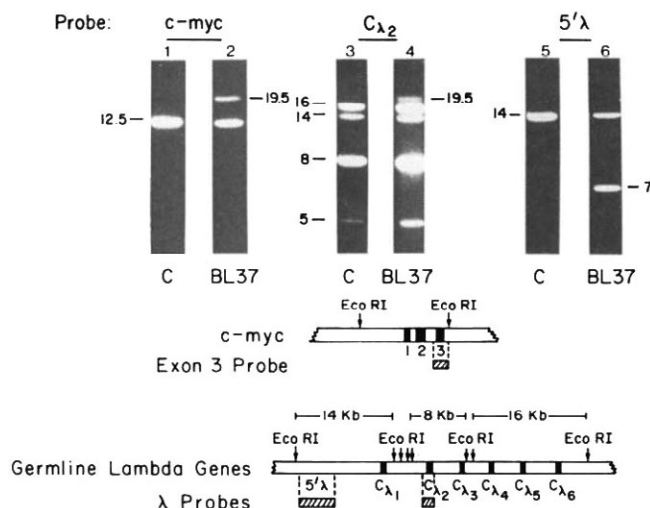


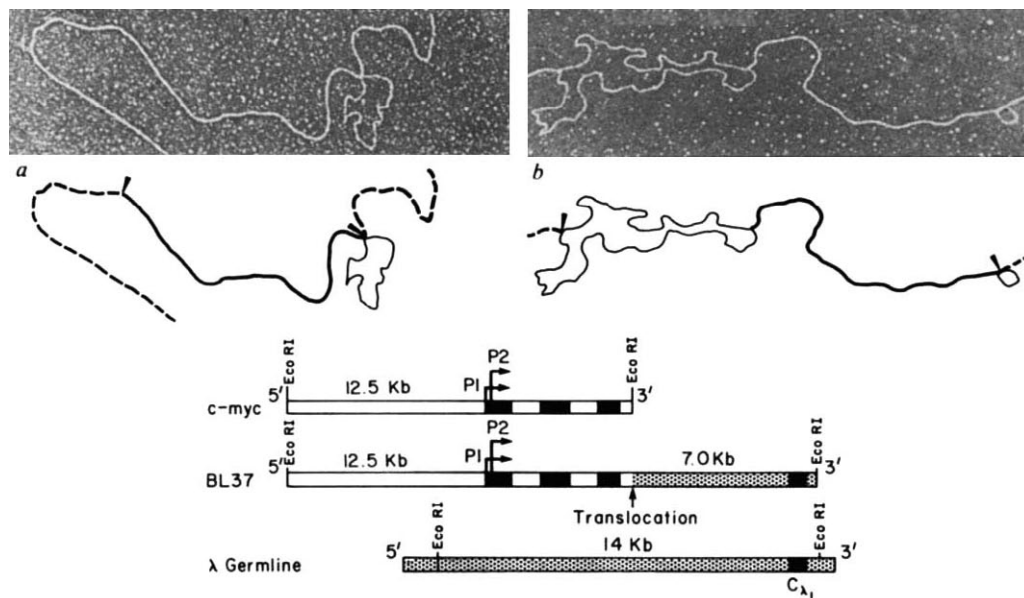
Fig. 1 The arrangement of *c-myc* and immunoglobulin λ gene segments in normal cells and the Burkitt's lymphoma BL37. Southern transfers²⁰ of *Eco*RI-digested genomic DNAs (10 μ g per lane) from normal human white blood cells, C, and a Burkitt's lymphoma cell line containing a t(8;22), BL37, were hybridized to ³²P-labelled DNA probes corresponding to exon 3 (*Cla*I-*Eco*RI) of the human *c-myc* gene (lanes 1, 2), human λ constant region 2 (*Bam*HI-*Bam*HI) (lanes 3, 4), and 5' flanking region of λ constant region 1 (*Eco*RI-*Hind*III) (lanes 5, 6). The probe locations used are shown in the diagram. The transfers were washed in 0.1 $\times$ SSC, 0.1% SDS at 52 $^{\circ}$ C and visualized by autoradiography. In addition to the 12.5-kb germ-line *Eco*RI band, the *c-myc* probe identifies a 19.5 kb band in the BL37. The λ constant region 1 probe identifies a non-germ-line 19.5 kb *Eco*RI fragment, as well as the 5, 8, 14 and 16 kb germ-line bands. The 19.5 kb and 7 kb non-germ-line *Eco*RI fragments were cloned into the λ vector Charon 4A (ref. 20) using the exon 3 *c-myc* probe and 5' flanking λ probe respectively.

and the appearance of the expected 7 kb reciprocal recombination product in the lane 6 indicates that the cross-over point lies between this 5' probe and the λ_1 gene. Both rearranged fragments were isolated by preparative gel electrophoresis and cloned in the λ phage vector Charon 4A²⁰.

The orientation of the two genes and the cross-over point of their recombination was established by comparing their structures with those of known germ-line *c-myc* and immunoglobulin genes^{9,16} by both heteroduplex mapping and restriction fragment map comparison. The heteroduplex structure shown in Fig. 2a was formed between the cloned 19.5-kb *Eco*RI fragment containing the rearranged *c-myc* gene and the germ-line *c-myc* gene. The structure has a 12.5-kb region of homology (representing virtually the entire 12.5-kb *Eco*RI germ-line DNA *c-myc* segment, see map) and a single-stranded substitution loop approximately 7 kb in length. The position of the substitution loop indicates that rearrangement has occurred 3' to the *c-myc* gene. When the same rearranged fragment is hybridized to a germ-line fragment of DNA derived from the λ_1 gene (maps in Figs 1, 2), a 7 kb length of homology is seen, as are two single-stranded regions of non-homology, 7.0 and 12.5 kb in length (Fig. 2b). Comparison of subclones derived from the cross-over region from the two germ-line genes and the products of their reciprocal recombination showed that the 12.5-kb portion of the 19.5 kb rearranged fragment has a restriction map identical to that of the germ-line *c-myc* gene and is then identical for 7 kb to the λ_{C1} immunoglobulin fragment (data not shown).

The orientation and breakpoints were finally established by determining the nucleotide sequence of the cross-over region in its germ line and rearranged configurations and the sequence of the cross-over region is shown in Fig. 3. With the exception of a 21 base pair (bp) segment of DNA derived from the λ_1 germ-line locus which is missing from the recombinants, the sequences at the breakpoints are entirely conserved over the sequenced regions. The data are all consistent with a translocation between a position 400 bases 3' to the poly(A) addition site of the *myc* gene and a point 5 kb 5' of the sequence

Fig. 2 Heteroduplex comparison of the rearranged *c-myc* gene derived from BL37 and germ-line *c-myc* and immunoglobulin λ genes. *a*, A recombinant phage clone containing the 19.5 kb *Eco*RI restriction fragment identified in Fig. 1 was heteroduplexed to a recombinant phage containing the 12.5 kb *Eco*RI *c-myc* fragment using alkali denaturation followed by renaturation in 50% formamide, 12.5 mM EDTA, 50 mM NaCl and 100 mM Tris pH 8.5 at 37 $^{\circ}$ C for 15 min. In addition to the heteroduplex formed between the vector phage arms, there is a region of homology (see arrows in graphic) that corresponds to nearly the entire 12.5 kb *Eco*RI *c-myc* DNA fragment. At a position 3' to the *c-myc* gene a 7 kb region of non-homology is seen. This heteroduplex structure is consistent with the 19.5-kb recombinant DNA phage clone containing nearly all of the 12.5 kb *Eco*RI fragment, including the three exons of *c-myc* that are normally located on chromosome 8. The structures of this 19.5 kb clone as well as the germ-line *c-myc* fragment are shown schematically. *b*, The phage containing the rearranged 19.5 kb *Eco*RI fragment BL37 was heteroduplexed to a phage containing a 15 kb segment of DNA that encodes the λ_1 gene that was derived from human fetal liver. The latter clone includes the 14 kb *Eco*RI fragment carrying λ constant region 1 as shown in the map. In this heteroduplex, a 7 kb homology is seen, as well as two single-stranded regions 12.5 kb and 7.0 kb in size. The position of the homology in this heteroduplex indicates that this library clone and the 19.5 kb clone share sequence homology for 7 kb 5' to λ constant region 1. The two clones diverge at this point, the region where the 19.5 kb clone carries sequence from the *c-myc* locus of chromosome 8. A small region of non-homology is seen (see arrow to the right), which corresponds to the distance the library clone extends 3' to the *Eco*RI site. These results are shown schematically.



12.5 kb *Eco*RI *c-myc* DNA fragment. At a position 3' to the *c-myc* gene a 7 kb region of non-homology is seen. This heteroduplex structure is consistent with the 19.5-kb recombinant DNA phage clone containing nearly all of the 12.5 kb *Eco*RI fragment, including the three exons of *c-myc* that are normally located on chromosome 8. The structures of this 19.5 kb clone as well as the germ-line *c-myc* fragment are shown schematically. *b*, The phage containing the rearranged 19.5 kb *Eco*RI fragment BL37 was heteroduplexed to a phage containing a 15 kb segment of DNA that encodes the λ_1 gene that was derived from human fetal liver. The latter clone includes the 14 kb *Eco*RI fragment carrying λ constant region 1 as shown in the map. In this heteroduplex, a 7 kb homology is seen, as well as two single-stranded regions 12.5 kb and 7.0 kb in size. The position of the homology in this heteroduplex indicates that this library clone and the 19.5 kb clone share sequence homology for 7 kb 5' to λ constant region 1. The two clones diverge at this point, the region where the 19.5 kb clone carries sequence from the *c-myc* locus of chromosome 8. A small region of non-homology is seen (see arrow to the right), which corresponds to the distance the library clone extends 3' to the *Eco*RI site. These results are shown schematically.

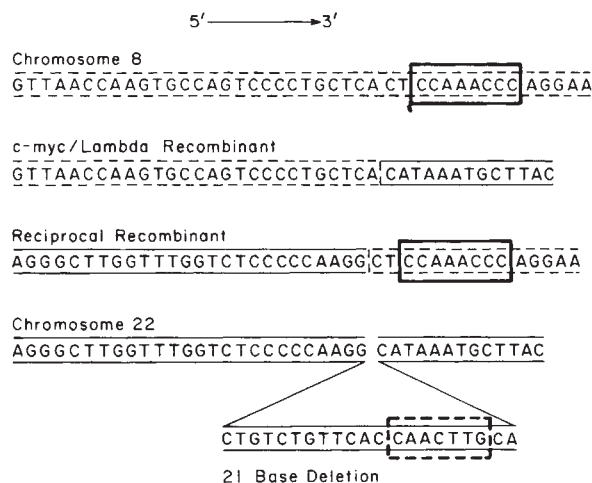


Fig. 3 Sequences of the regions involved in the 8;22 translocations in BL37 were determined by dideoxy sequence analysis²⁶. Four aligned sequences are shown, each of which represents the DNA strand which bears the coding sequences of the genes. The upper sequence represents germ-line chromosome 8. The second sequence is the recombinant which bears the *myc* and λ genes. The third sequence is the reciprocal translocated product and the fourth sequence represents the germ-line chromosome 22. The 21 bases deleted in the translocation event are indicated separated from the adjacent sequence. The CCAAACCC sequence described in the text is boxed with a solid line and the YAGGTTG sequence boxed with a dashed line.

that has been identified as the J region of λ_{C1} (N. Berliner, unpublished).

In the other translocations investigated thus far, the *c-myc* gene is often joined to one of the heavy-chain switch region signals^{4,6,9-13}. It is, therefore, attractive to think that the switch recombination system catalyses the Burkitt-type translocation. Analysis of the structure of the cross-over point of the BL37 translocation has allowed us to compare the structure of the substrates and products of the recombination event. Several hundred nucleotides of sequence in this region from both germ-line *c-myc* and immunoglobulin genes have been examined and extensive regions of switch-like homology have not been found. Therefore, the situation in the BL37 cell line, in which neither partner provides an extensive switch signal, makes such a mechanism less likely. It is, therefore, worth noting that the sequence, YAGGTTG, which has been found 8–16 nucleotides 5' of many normal heavy-chain switching events²¹, occurs in 6/7 consensus form 11 bases away from one of the breakpoints on chromosome 22. This sequence does not appear in the products of the translocation since it occurs within the 21 bp segment that is deleted (Fig. 3). Also, an octanucleotide, CCAAACCC, occurs very close to the cross-over point in the *c-myc* (chromosome 8) segment (Fig. 3). This sequence also flanks the recombination site in the translocation that occurs 5' to the *c-myc* gene in the Burkitt line BL22⁹. We note that a sequence which differs by only a single base from this is present in reverse complement form adjacent to the breakpoint in a murine plasmacytoma reported recently²². It will require the characterization of further examples before we will be able to evaluate the significance of these observations.

One clue to a potentially important regulatory feature of the *c-myc* gene comes from comparative studies of its normal structure⁹. The *c-myc* gene is encoded in three exons, the first of which is a 550 bp untranslatable leader sequence that is tightly conserved in sequence between mouse and human species. This region encodes two promoter sequences that define two transcription initiation sites about 160 bp from one another (map,

Fig. 4 Comparative analysis of the mRNA derived from BL37 and other Burkitt's lymphoma and lymphoblastoid cell lines. Total RNA was made²⁷ from Burkitt's lymphomas BL37, J1, Ly67 and Ly47 and the lymphoblastoid cell line IARC 100 and used for RNA blotting and S_1 nuclease analysis. RNA blots²⁸ from Burkitt's lymphomas J1 and BL37, both κ light-chain producers, were probed with a DNA (*EcoRI-EcoRI*) probe containing the κ constant region sequence. RNA from both cell lines hybridized specifically to give one band on the blot (see C_k probe). When J1 and BL37 RNA, as well as RNA from a λ -producing Burkitt's lymphoma, Ly67, was probed with a λ constant region, only the RNA from the Ly67 hybridized to the probe (see $C_{\lambda 2}$ probe). Transcription of the *c-myc* gene was determined in BL37 as well as IARC-100 and Burkitt's lymphoma Ly47, a λ producer, by probing with the second coding exon of *c-myc* (see Fig. 1). All three cell lines produce a 2.3 kb *c-myc* specific RNA, but the amount of *c-myc* RNA in BL37 appears higher than in IARC 100 (see *c-myc* probe). To control for the amount of RNA loaded in each lane, the same RNA blot was rehybridized with a β_2 -microglobulin probe²⁹. All three cell lines produce a 1.0 kb β_2 -microglobulin specific RNA. By using the β_2 -microglobulin specific hybridization as a control for RNA concentration, densitometric scans showed that BL37 produces 15-fold more *c-myc* RNA than IARC 100. To determine the point of initiation of transcription of the *c-myc* gene in BL37, an 860-bp *PvuII-PvuII* M13 mp9 clone containing the two promoters of transcription was labelled *in vitro* with ³²P-dATP by primer extension⁹. The single-stranded probe was allowed to anneal to 10 μ g of tRNA and 10 μ g of total RNA from BL37 and IARC 100 and then digested by 750 units of S_1 nuclease essentially as described by Berk and Sharp²³. The digestion products were electrophoresed on an 8 M urea 5% acrylamide and visualized by autoradiography (see *c-myc/S1*), tRNA alone showed no protection of the probe (data not shown).

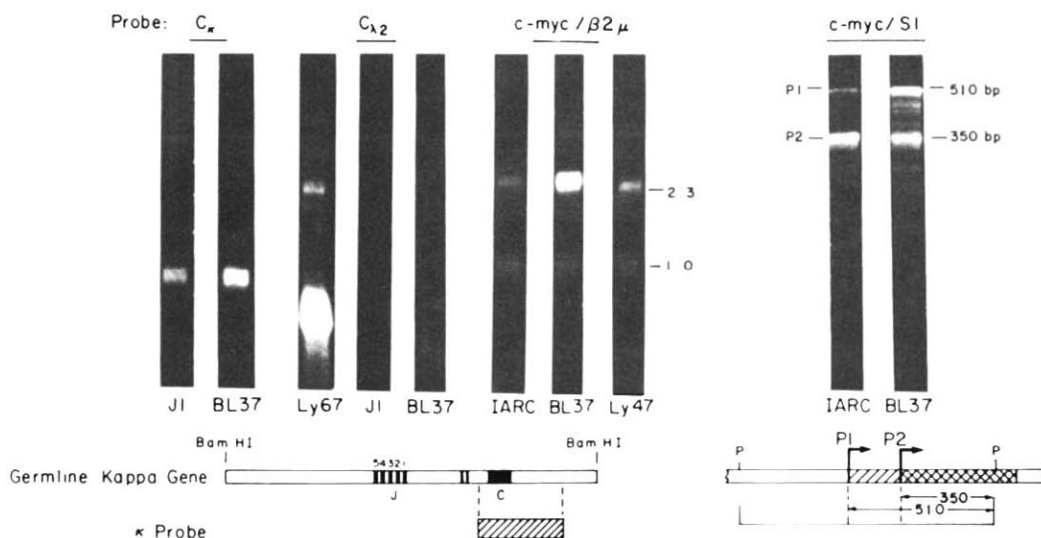


Fig. 4). Studies carried out in EBV-immortalized lymphoblastoid cell lines and non-transformed fibroblasts indicate that products derived from the use of the second promoter, P₂ (Fig. 4), are consistently represented among the stable *c-myc* transcripts of these cells at a higher level than transcripts derived from the first promoter, P₁. The fixed, approximately fourfold excess of P₂ transcripts in untransformed cells suggests the existence of a regulatory programme that maintains this ratio²⁴.

With these features in mind, we have used RNA blot hybridization to examine the immunoglobulin light chain and *c-myc* transcripts in several Burkitt's lymphoma cell lines including BL37 and a lymphoblastoid cell line. The results are shown in Fig. 4. The first two sets of panels in Fig. 4 show that the BL37 cell line produces large amounts of κ mRNA, but only trace amounts of λ RNA. Control Burkitt cell lines that produce only κ (J1) and only λ (Ly67) mRNAs are shown for comparison. The third set of three panels compares the *c-myc* transcript present in a lymphoblastoid cell line (IARC-100) with that in the BL37 cell line and another Burkitt cell line, IARC-Ly47. The level of β_2 -microglobulin mRNA is determined in each cell line to control for the amount of mRNA added to each lane. Although the amount of β_2 -microglobulin is approximately equivalent in each lane, the level of *c-myc* transcript is about 15-fold higher in the BL37 cell line. In contrast, the *c-myc* RNA is only slightly increased (1.4-fold) in the Burkitt line Ly47 when compared with the non-malignant IARC-100 cell line.

A more revealing analysis is one that distinguishes between the products of the two *c-myc* promoters. Such an experiment, carried out using the S₁ nuclease technique²³, is shown in lanes 9 and 10 of Fig. 4 (lanes *c-myc*/S₁). As with other lymphoblastoid cell lines, the IARC-100 cell line has a predominant transcript that arises from the second promoter, P₂, which is represented in about fourfold excess over that which arises from the first promoter, P₁. In contrast, the two *c-myc* promoters P₁ and P₂ are both extensively used in BL37 indicating an activation of the *myc* gene in this cell line similar to that we have observed in other Burkitt-derived lines²⁴.

The orientation of the *c-myc* gene on chromosome 8 may be deduced from the data presented by Dalla-Favera *et al.*²⁵ to be centromere-(5'-*c-myc*-3')-telomere. The genes on the recombinant chromosome in BL37 are arranged 5'-*c-myc*-3'-5'- λ 1-3' (tail-to-head). Since the breakpoint on chromosome 8 is 3' to the *c-myc* gene, the gene itself must be associated with its original centromere in the recombinant chromosome. Therefore, the germ-line orientation of the λ genes is established by the structure of the translocation product and must be centromere-(5'- λ 1-3')-telomere. Since all the λ genes are encoded on the same strand of DNA, one can predict that all simple translocations between chromosomes 8 and 22 will place the *c-myc* and λ immunoglobulin genes in a tail-to-head orientation. It is possible that some chromosomes will rearrange such that the λ gene will in fact be 5' to the *c-myc* gene in a tail-to-head orientation and the *myc* gene could be affected by upstream λ gene promoters or enhancers.

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Novel mannosidase inhibitor blocking conversion of high mannose to complex oligosaccharides

Ulrike Fuhrmann*, Ernst Bause†, Günter Legler† & Hidde Ploegh*§

Institute for *Genetics and †Biochemistry, University of Cologne, Weyertal 121, 5000 Cologne 41, FRG

Many secretory and membrane proteins are glycoproteins carrying asparagine-linked (*N*-linked) oligosaccharides. There are two types of *N*-linked glycans, referred to as high-mannose and complex type, respectively. Biosynthesis of *N*-linked glycans of the complex type proceeds via a high-mannose intermediate. After the initial transfer of a high-mannose oligosaccharide with the composition (Glc)₃(Man)₉(GlcNAc)₂ from a lipid carrier to the nascent polypeptide chain, trimming reactions take place. Trimming glucosidases remove the glucose residues quantitatively and mannosidases IA/B and II can remove all but three mannose residues. After trimming, terminal sugars such as *N*-acetylglucosamine, galactose, sialic acid and fucose may be added and result in the conversion to a glycan of the complex type¹. Because suitable inhibitors were lacking, it was difficult to assess the importance of the trimming reactions for proper intracellular traffic, modification reactions other than the addition of terminal sugars, or as regulatory steps in glycoprotein processing. Here we describe the action of 1-deoxymannojirimycin (1,5-dideoxy-1,5-imino-D-mannitol, dMM; Fig. 1) on the biosynthesis of IgM and IgD. dMM is the mannose analogue of 1-deoxynojirimycin (dNM; Fig. 1), itself a glucosidase inhibitor²⁻⁴. We present evidence that dMM is a mannosidase inhibitor. *In vivo* dMM inhibits the equivalent of the mannosidase IA/B activities and blocks conversion of high-mannose to complex oligosaccharides. It is the first such inhibitor to be reported. Interference with the biosynthetic pathway of *N*-linked glycans could prove to be a powerful way to manipulate carbohydrate structure *in vivo*.

The experimental system consists of hybridoma cells producing IgM⁵ and IgD⁶ specific for the hapten (4-hydroxyl-3-nitrophenylacetyl, NP). After preincubation with inhibitor, these cells were pulse labelled for 10 min with ³⁵S-methionine and then chased with non-radioactive methionine in the continued presence of inhibitor. The inhibitors used were dMM and, for comparative reasons, the mannosidase II inhibitor swainsonine^{7,8} (SW; Fig. 1). At different times of chase samples were taken and immunoglobulin was isolated from detergent lysates of cells (cytoplasmic immunoglobulin) or culture supernatants

§ To whom reprint requests should be sent.

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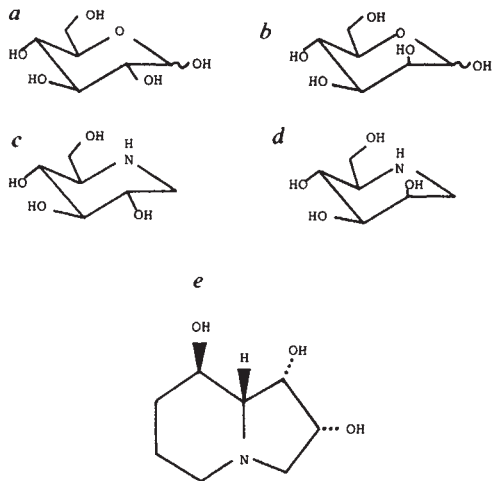


Fig. 1 Structures of glucose, *a*, mannose, *b*, the corresponding sugar analogues 1-deoxynojirimycin, *c* and 1-deoxymannojirimycin, *d* and the mannosidase II inhibitor⁸ swainsonine, *e*.

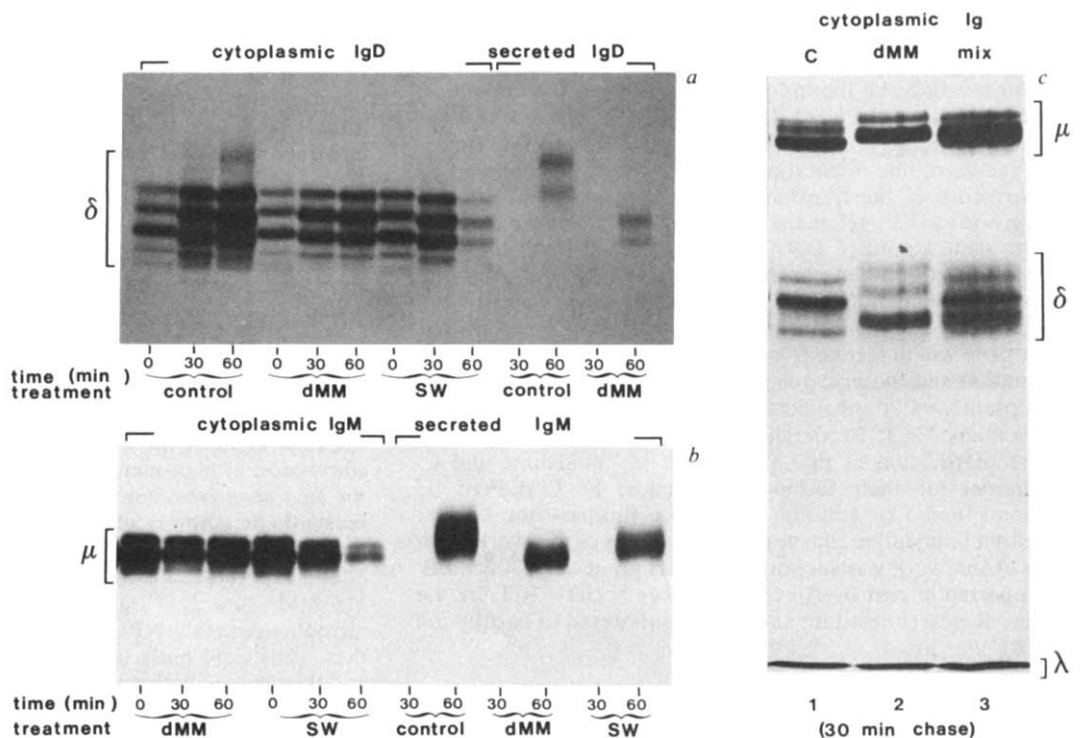
(secretory immunoglobulin) by incubation with an affinity support (Fig. 2). For control cells and SW-treated cells the cytoplasmic immunoglobulin shows a steady decrease in apparent molecular weight (M_r) with time in pulse-chase experiments of this type^{3,9}, due to oligosaccharide trimming (Fig. 2*a*). On secretion an observed increase in M_r is caused by the addition of terminal sugars⁹ (Fig. 2*a*). The M_r of cytoplasmic immunoglobulin from SW-treated and control cells is indistinguishable at each corresponding chase time as reported earlier³. Immunoglobulin from dMM-treated cells has a higher M_r than that from control cells, suggesting the inhibition of trimming reactions. In Fig. 2*b* these differences in M_r can be clearly demonstrated in a mixing experiment. dMM does not inhibit secretion of IgM or IgD (Fig. 3), as opposed to dNM which was shown to inhibit

secretion of IgD but not IgM³. The analysis of IgD precursors is complicated by the fact that differentially *N*-glycosylated membrane and secretory IgD are present simultaneously^{6,9}. This explains the multitude of δ -specific bands in Figs 2 and 3. In dMM-treated cells the secreted δ chains are indistinguishable in M_r from the middle two bands present in the cytoplasmic samples (Fig. 2*a*). Similarly, no difference in M_r was observed for secretory IgM and cytoplasmic IgM at 60 min in dMM-treated cells (Fig. 2*c*). It is possible that in the presence of dMM, terminal modifications can no longer occur because removal of mannose residues is incomplete. In SW-treated cells terminal modifications do take place, but to a lesser extent than in control cells, as evidenced by a lower M_r of secreted immunoglobulin. This observation is consistent with the inhibitory action of SW on mannosidase II which does not allow all oligosaccharide antennae to be terminally modified^{3,10}.

The results obtained with dMM suggest that no terminal modifications occur and that the target of dMM inhibition *in vivo* acts before that of SW. Such a situation would be consistent with inhibition of mannose removal, that is, inhibition of the mannosidase IA/B activity. If correct, a (Man)₅(GlcNAc)₂ structure, which is endoglycosidase H (EndoH) sensitive¹¹, would be expected to accumulate and be present on immunoglobulin secreted by dMM-treated cells. Therefore, no incorporation of terminal sugars such as galactose and sialic acid should occur. Moreover, the oligosaccharides should be of a size consistent with this hypothesis and should remain sensitive to EndoH. Therefore the following experiments were carried out.

IgM- and IgD-producing cells were mixed and preincubated with inhibitor for 4 h. These cells were then labelled with ³H-galactose, and a small aliquot was labelled in parallel with ³⁵S-methionine. Immunoglobulin was prepared from the culture supernatants and analysed by SDS-polyacrylamide gel electrophoresis (PAGE). Secreted immunoglobulin from SW-treated and control cells was readily labelled with ³H-galactose (Fig. 3*a*), whereas no label was incorporated into immuno-

Fig. 2 Pulse-chase analysis of the biosynthesis of IgD (*a*, *b*) and IgM (*b*, *c*). Cells (6×10^6 per sample) were preincubated with inhibitor (dMM or SW, at 1 mM or 10 μ g ml⁻¹ respectively) for 1 h, pulse-labelled with 50 μ Ci of ³⁵S-methionine per sample and chased with non-radioactive methionine in the continued presence of inhibitor. SW was a gift of Drs Vosbeck and Elbein, dMM was a gift of Drs Kinast and Truscheit; a novel scheme for the synthesis of dMM has been developed and will be published elsewhere¹⁵. Samples were taken for isolation of immunoglobulin at the times indicated in the figure. Detailed procedures for labelling of cells isolation of NP-binding immunoglobulin on affinity matrices and analysis by SDS-PAGE/fluorography have been described^{3,6,9}. Except for *b*, only the corresponding heavy chain regions of the autoradiograms are shown. Immunoglobulin was isolated or from detergent extracts of cells (cytoplasmic immunoglobulin) or from culture supernates (secreted immunoglobulin). C, control; dMM, 1-deoxymannojirimycin; SW, swainsonine. For *b*, approximately equal amounts of IgM and IgD biosynthetic precursors were mixed and co-electrophoresed. Cytoplasmic immunoglobulin for 30 min is shown for control cells (lane 1), dMM-treated cells (lane 2) and a mixture of the samples from lanes 1 and 2 (lane 3).



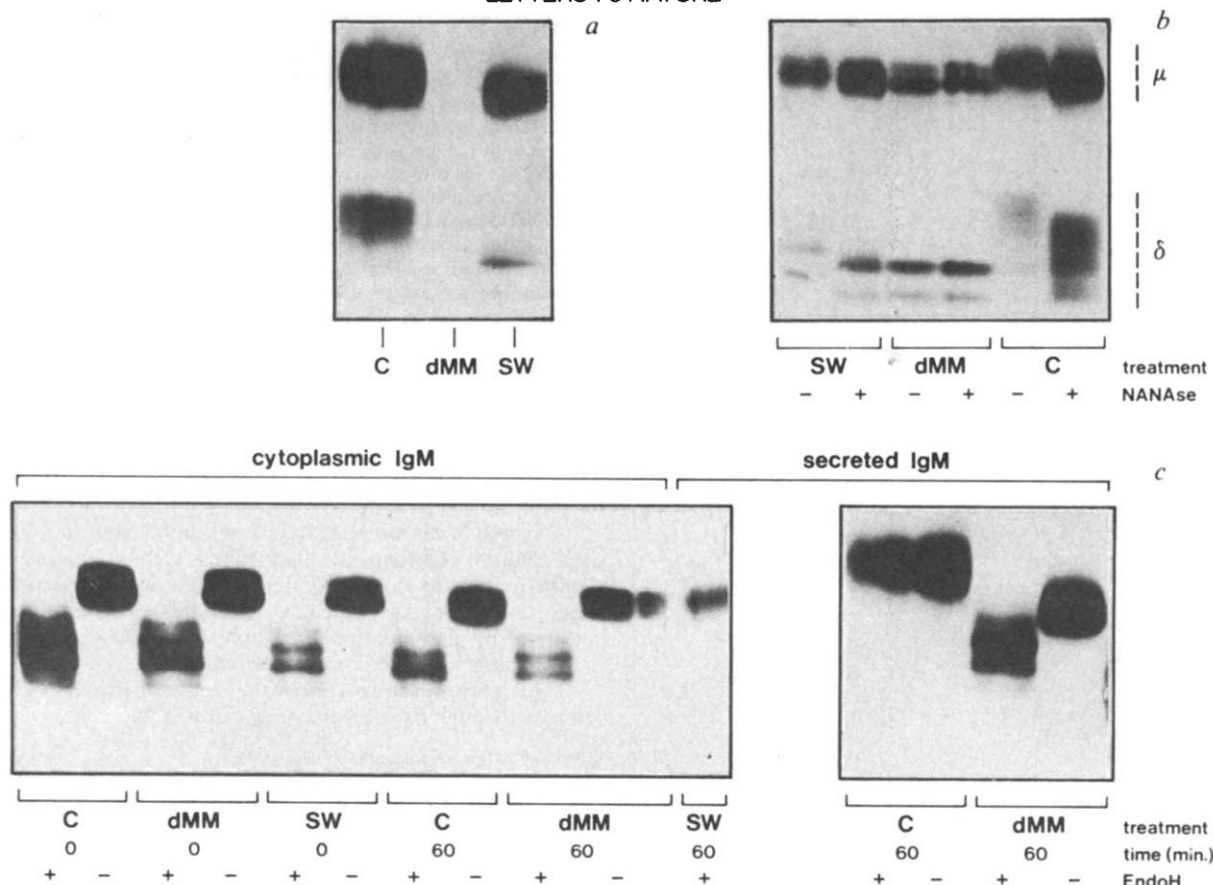


Fig. 3 Analysis of IgM and IgD biosynthesis by sugar labelling, neuraminidase and endoH treatment. *a*, A mixture of IgD- and IgM-producing cells (8×10^6 cells ml^{-1} , final volume 1.25 ml) were preincubated with dMM (1 mM) or SW ($10 \mu\text{g ml}^{-1}$) for 4 h. A 1-ml aliquot was then labelled with ^3H -galactose as described elsewhere³. Immunoglobulin was isolated from the culture supernatant and displayed by SDS-PAGE as in Fig. 2. *b*, The remainder of the cells (250 μl) were labelled with ^{35}S -methionine after transfer into methionine-free medium containing inhibitor. Immunoglobulin was isolated from the supernatant and analysed as in Fig. 2. Neuraminidase digests were prepared as described elsewhere⁹ and were run on the same gel. *c*, Samples obtained from ^{35}S -methionine-labelled samples prepared as described in Fig. 2, were taken at the times indicated and digested with EndoH as described elsewhere⁹.

globulin secreted by dMM-treated cells. Synthesis and secretion of immunoglobulin were not inhibited, as is evident from the ^{35}S -methionine labelling experiment carried out in parallel: similar quantities of immunoglobulin, as verified by quantitation of trichloroacetic acid precipitable material (data not shown) were secreted by SW-treated, dMM-treated and control cells (Fig. 3*b*). The ^{35}S -methionine-labelled samples were subjected to digestion with neuraminidase (NANase). Removal of sialic acid residues is accompanied by a decrease in M_r (ref. 9), as is observed for both control and SW-treated samples. Immunoglobulin from dMM-treated cells was not susceptible to NANase (Fig. 3*b*). Further proof of inhibition of conversion of high mannose to complex type oligosaccharides was derived from digestion with EndoH of ^{35}S -methionine-labelled samples. Cytoplasmic immunoglobulin precursors, regardless of time or treatment, were sensitive to EndoH (Fig. 3*c*). Immunoglobulin secreted by SW-treated and control cells was resistant to EndoH, whereas IgM secreted by dMM-treated cells was as sensitive to EndoH as cytoplasmic IgM (Fig. 3*c*).

Conclusive evidence for the inhibition of high mannose to complex type oligosaccharide conversion comes from the analysis of EndoH-released oligosaccharides, obtained from dMM-treated cells labelled with ^{14}C -mannose. After digestion of the labelled immunoglobulin with EndoH the size of the released oligosaccharides was determined by gel filtration in comparison with standards of known structure. The elution profile of these oligosaccharides on a Biogel P4 column, as well as that of marker oligosaccharides, is shown in Fig. 4. The major peak of EndoH-released IgM oligosaccharides (III, Fig. 4*b*) eluted at a position identical to that of a $(\text{Man})_9(\text{GlcNAc})$ marker (I, Fig. 4*a*). The

absence of radioactivity in the void volume again indicates that all oligosaccharides on IgM from dMM-treated cells were fully susceptible to EndoH. Approximately one-quarter of the released oligosaccharides was found to elute at a position corresponding to that of $(\text{Man})_8(\text{GlcNAc})$ (IV, Fig. 4*b*), probably the consequence of incomplete inhibition of mannose removal in our experimental conditions. The peaks from the Biogel P4 column were recovered and analysed in parallel by TLC in conditions that allow resolution of the various oligosaccharides (Fig. 4*c*). The major peak (III) possessed an R_F identical to that of the $(\text{Man})_9(\text{GlcNAc})$ marker (II). The minor peak had a mobility consistent with a $(\text{Man})_8(\text{GlcNAc})$ structure (IV). Thus the combined data show that dMM treatment results in the retention of the high mannose configuration.

1-Deoxymannojirimycin is the first inhibitor to be described that inhibits *in vivo* conversion of high mannose to complex type oligosaccharides by blocking the equivalent of a mannosidase IA/B activity. Substances have now been identified that inhibit *in vivo* trimming glucosidases^{3,4,12,13}, mannosidase II^{7,8}, and, here, also mannosidase IA and/or IB. Earlier results obtained with tunicamycin^{3,9} and 1-deoxymannojirimycin³ showed that elimination of *N*-linked glycosylation altogether or manipulation of glucosidase trimming may have different effects on secretion of immunoglobulin. However, we found no evidence for an inhibitory effect of dMM on secretion of IgM or IgD. The observation that dMM, the mannose analogue of dNM, itself a potent glucosidase inhibitor, is a mannosidase inhibitor suggests that the reaction mechanisms for trimming glucosidases and mannosidase reactions are similar. Both may be postulated to proceed through a cationic reaction intermediate².

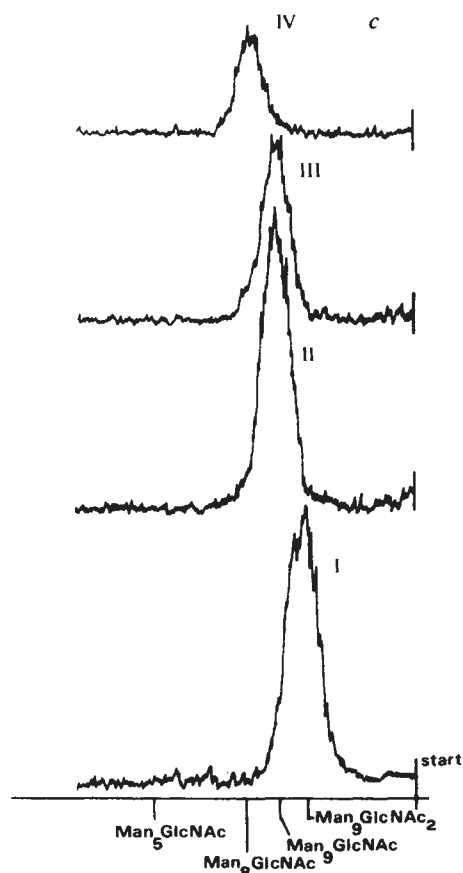
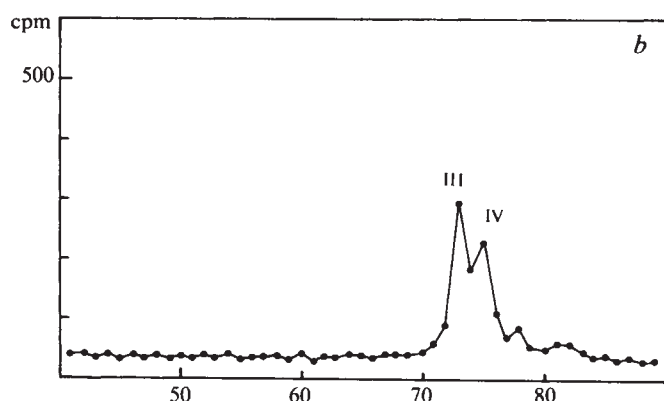
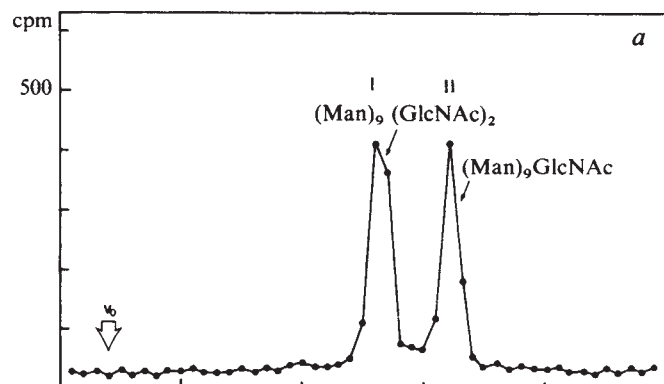


Fig. 4 Gel filtration of ^{14}C -mannose-labelled oligosaccharides obtained from IgM secreted by dMM-treated cells. IgM-producing cells were labelled with ^{14}C -mannose ($50\ \mu\text{Ci}$) for 8 h in the presence of 1 mM dMM. IgM was isolated from the culture supernatant as in Fig. 2 and digested with EndoH as described elsewhere⁹ but with the inclusion of 0.1% SDS after 3 h digestion. The digest was applied directly to a Biogel P4 (200–400 mesh) column ($180 \times 1.2\ \text{cm}$) and eluted with 1 M acetic acid. Fractions of 1.32 ml were collected and 200 μl aliquots were counted by liquid scintillation counting. Recovery was better than 90%. The included volume of the column, as determined by chromatography of ^{14}C -glucose, was at fractions 98–102. The void volume is indicated by an open arrow. *a*, Elution profile of marker oligosaccharides $(\text{Man})_9(\text{GlcNAc})_2$, I, and $(\text{Man})_9(\text{GlcNAc})$, II, prepared as described elsewhere⁴. *b*, Elution profile of IgM-derived oligosaccharides after digestion with EndoH. *c*, TLC of: I (fractions 66–67); II (fractions 72–74); and IV (fractions 75–76) from *b* in silica gel. The plate was developed twice with *n*-propanol:acetic acid:water (3:3:2). Radioactivity profiles obtained by scanning are shown. Peaks II and III have identical R_F values ($(\text{Man})_9(\text{GlcNAc})$).

Carbohydrate moieties have been implicated in a variety of recognition phenomena, ranging from cellular aggregation¹⁴ to participation in embryonic development and immune recognition. Such problems can now be addressed in a more refined manner by manipulation of carbohydrate structure through use of oligosaccharide trimming inhibitors.

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A new myosin fragment: visualization of the regulatory domain

Donald A. Winkelmann, Sheri Almeda, Peter Vibert & Carolyn Cohen

Rosenstiel Center, Brandeis University, Waltham, Massachusetts 02254, USA

Many of the functional domains of the myosin molecule have been defined by the use of proteolytic enzymes. Major fragments that retain enzymatic or assembly properties have been prepared by cleavage in the rod to form heavy meromyosin (HMM) and light meromyosin (LMM)¹ or at the head-rod junction to form S-1 and rod^{2,3}. Limited tryptic digestion of vertebrate skeletal myosin S-1⁴ indicates that the head contains three major regions: an amino-terminal nucleotide binding domain of molecular weight (MW) 25,000^{5,6}, a central domain of MW 50,000 and a carboxyl domain MW 20,000; the latter two are both able to bind to actin^{7,8}. Tryptic digestion of scallop S-1 has also been used to isolate a head fragment MW 14,000 associated with both types of scallop light chains⁹. Here we report that myosin from vertebrate (chicken and rabbit skeletal) and molluscan (scallop adductor) striated muscles is cleaved in an unusual way with an enzyme from *Pseudomonas aeruginosa*^{10–12}. This bacterial protease (designated Ps-1) does not cleave myosin at the head-rod junction or in the rod;

instead, Ps-1 splits the myosin heavy chain within the head, yielding a complete rod joined to the 20,000-MW head domains. The scallop regulatory and essential light chains remain associated with this fragment. We examined this new fragment by electron microscopy; the rods bear two 'nubs' about 100 Å long, which appear to correspond morphologically to the neck region of the myosin molecule^{13,14}.

In contrast to trypsin¹, chymotrypsin³ or papain², Ps-1 cleaves myosin initially at the 75,000–20,000 junction in the head so that a new, larger rod fragment (MW 140,000) and a head fragment (MW 75,000) are produced (Fig. 1). The rod contains the complete rod heavy chain (MW 120,000) together with an additional 20,000-MW peptide derived from the head (Figs 2, 3). The reactive sulphhydryls (SH-1 and SH-2)¹⁵ are retained on this 140,000 fragment (data not shown). The associated myosin light chains also show limited cleavage by Ps-1: the two larger light chains (LC1 and LC2) of the vertebrate myosins and the regulatory light chain (RLC) of scallop myosin each lose fragments with molecular weight of about 1,000 (Fig. 1).

The 75,000-MW head fragment is further cleaved by Ps-1 to 25,000 and 50,000 fragments which correspond respectively to the nucleotide^{5,6} and actin⁷ binding domains identified by tryptic digestion of S-1 (Fig. 2). These head domains from vertebrate striated myosins are then stable even after long incubation periods with Ps-1 (48 h). This selectivity of Ps-1 contrasts with that of trypsin which splits myosin heads into similar domains, but degrades these fragments on longer incubation¹⁶. Scallop myosin appears, however, to be particularly sensitive to proteolysis, since the head fragments are further digested by Ps-1 (Fig. 1). This property does not appear to be related to the light chain-dependent regulation of this molecule¹⁷. Vertebrate smooth muscle myosin (turkey gizzard) also exhibits a form of myosin-linked regulation¹⁸, and upon digestion with Ps-1 yields

stable head domains of slightly differing molecular weights (47,000 and 28,000) and a 140,000 rod fragment (data not shown).

The sensitivity of the scallop head to digestion permits the new rod fragment to be isolated from this myosin. Most of the degraded scallop head fragments remain soluble at low ionic strength, whereas the scallop Ps-1 rod fragment, with both the regulatory and essential light chains still associated, precipitates in these conditions (Fig. 3b). In contrast, all of the heavy and light chain fragments remain associated in Ps-1-digested chicken and rabbit myosin on low ionic strength precipitation or on binding to F-actin.

Electron microscopy of this new scallop rod fragment using rotary shadowing shows predominantly a population of rods terminating in two nubs, each about 100 Å long (Fig. 4b). Some variability is found in the appearances of the nub structures, which may reflect the small size and flexibility of this region in myosin^{14,19}. Although the head fragments remain associated with the rod in Ps-1-digested vertebrate myosins, shadowed preparations occasionally show molecules with one-nub/one-head and even two nubs (Fig. 4d, e).

These nubs appear to correspond morphologically to the 'neck' region of the myosin head. Myosin light chains have been localized in this region of both scallop adductor myosin¹⁴ and chicken pectoralis myosin²⁰. Moreover, it is likely that the 14,000-MW light chain binding fragment recently isolated from scallop S-1⁹ corresponds to a portion of this region. These results, together with the findings presented above, suggest that the neck region of myosin is comprised of the 20,000-MW domain with the associated light chains. We have called this region the 'regulatory domain' because the functional significance of this part of the molecule is established both for scallop^{21,22} and vertebrate smooth muscle myosin^{18,23,24}, and has recently also been suggested for vertebrate striated myosin²⁵. In this connection, it is likely that the precise digestion pattern

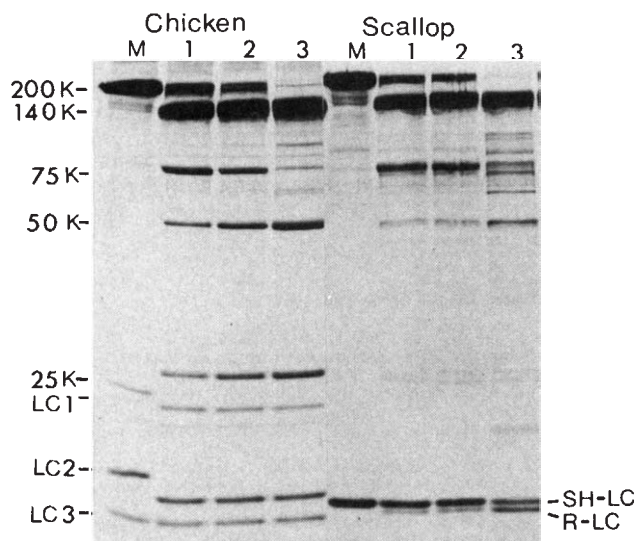


Fig. 1 Myosin fragments obtained by *Pseudomonas* protease (Ps-1) digestion. Digests of chicken pectoralis and scallop adductor myosin were analysed by Laemmli²⁷ SDS-polyacrylamide gel (12.5%) electrophoresis. M, myosin; lane 1, 1/200 dilution of protease; lane 2, 1/100; lane 3, 1/10. Note the formation of the stable fragments of MW 140,000 for both chicken and scallop myosin. The 75,000-MW fragment is cleaved to 25,000 and 50,000 fragments which are stable fragments from chicken pectoralis myosin; however, these fragments from scallop myosin are further degraded. LC1 and LC2 of the vertebrate myosins and the scallop regulatory light chain (RLC) each lose fragments of about MW 1,000. The rabbit psoas myosin digest pattern was identical to the chicken myosin pattern. Ps-1 is a serine protease with a narrower specificity than trypsin which was isolated from *Pseudomonas aeruginosa*¹²; it has been purified and is being characterized (S.A. and C.C., in preparation). Myosin (5 mg ml⁻¹, 0.5 M NaCl, 0.02 M NaH₂PO₄ pH 7.5, 1 mM MgCl₂) was digested for 24 h (4°C) with various dilutions of purified Ps-1 stock (0.3 mg ml⁻¹).

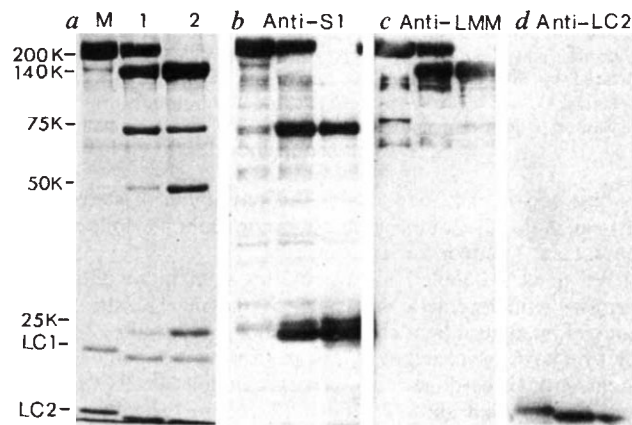


Fig. 2 Immunochemical identification of the fragments obtained by Ps-1 digestion of chicken myosin. *a*, Myosin, M, and the Ps-1 fragments obtained by digestion at two protease concentrations (lane 1, 1/100 dilution; lane 2, 1/10) were electrophoresed on a Laemmli SDS-polyacrylamide gel (11%). This pattern was transferred to nitrocellulose paper and these replicas were probed for specific antibody binding to the myosin fragments using monoclonal antibodies which react with defined regions of the myosin heavy and light chains²⁰. *b*, Anti-S-1 (12C5.3) recognizes an epitope in the amino-terminal fragment (MW 75,000) of S-1. This antibody identifies the band (MW 75,000) as an S-1 fragment which breaks down to the amino-terminal 25,000-MW fragment and a 50,000-MW fragment. *c*, Anti-LMM (5C3.2) identifies an epitope that maps to the carboxyl-terminus of the myosin rod. This antibody identifies the 140,000-MW fragment as a larger form (~20,000) of the myosin rod. *d*, Anti-LC2 (7C10.2) identifies the LC2 fragment obtained by Ps-1 digestion. Antibody binding to the nitrocellulose replicas was detected with ¹²⁵I-labelled rabbit anti-mouse F(ab')₂ and autoradiography. The SH-LC and RLC fragment produced by Ps-1 cleavage of scallop myosin have been similarly identified using polyclonal rabbit antisera²⁸ (data not shown).

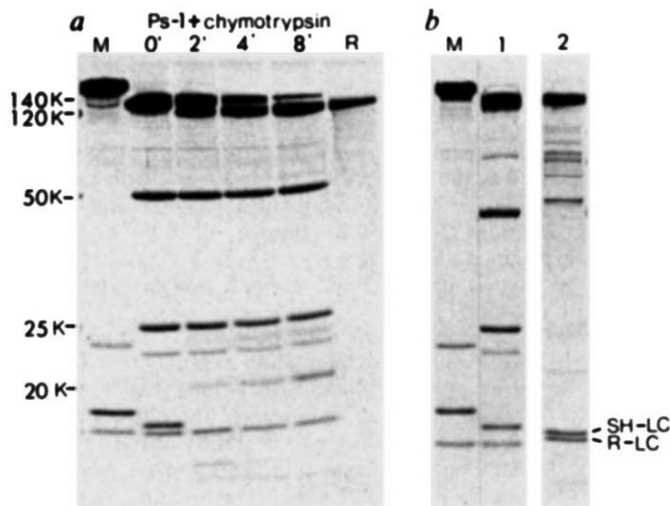


Fig. 3 *a*, The 20,000-MW head fragment can be released from the 140,000-MW Ps-1 fragment by chymotryptic digestion of Ps-1 cleaved myosin. After Ps-1 cleavage, chicken pectoralis myosin filaments (5 mg ml⁻¹, 0.12 M NaCl, 0.02 M NaH₂PO₄ pH 7.0, 1 mM EDTA) were treated with α -chymotrypsin (50 μ g ml⁻¹) for 0–8 min in conditions that selectively cleave the head–rod junction³. The 140,000-MW Ps-1 rod is reduced by chymotryptic digestion to a 120,000-MW fragment which co-migrates with the papain rod fragment (R), and the 20,000-MW S-1 peptide appears simultaneously. This 20,000-MW peptide has been shown to contain the reactive sulphhydryl (SH-1) by selective labelling of this cysteine with IAEDANS¹⁶ (data not shown). *b*, SDS-gel electrophoresis of Ps-1 digested chicken and scallop myosin after precipitation by dialysis against low-salt buffer. M, chicken pectoralis myosin; lane 1, Ps-1 digested chicken myosin heavy and light chain fragments remain associated in the washed pellet; lane 2, washed low-salt pellet of scallop digest; bands identified immunochemically as the scallop light chains remain associated with the rod fragment. Variable amounts of the head fragments were present in the rod pellet in different experiments, but in all cases the nub appearance predominated. Digested chicken and scallop myosin were precipitated by dialysis against low salt buffer (40 mM NaCl, 5 mM NaH₂PO₄ pH 6.7, 1 mM MgCl₂, 1 mM dithiothreitol), and the washed pellets were analysed by Laemmli SDS-polyacrylamide gel (12.5%) electrophoresis.

of this region may depend on the physiological state of the myosin molecule (for example, with or without phosphorylation, bound Ca²⁺ and/or nucleotide).

We need a detailed picture of this domain to understand myosin–actin interactions as well as the mechanism of Ca²⁺ control by myosin light chains. The elongated shape of the nub that we have visualized by rotary shadowing is in general agreement with three-dimensional reconstruction results of Vibert and Craig¹³ that show a long neck region for myosin heads containing light chains. The precise length and shape of the regulatory domain in the intact molecule are not known, but these results suggest that this domain may span about half the length of the head^{14,19}. We have yet to determine the relation between this picture of the head and cross-linking experiments with myosin S-1 and actin^{7,8} which suggest that there is a second weak actin-binding site localized in the 20,000-MW domain of the head. A second site has, in fact, been tentatively identified as a ‘finger’ of low density near an adjacent actin monomer in three-dimensional reconstructions of decorated thin filaments²⁶. Studies are in progress to determine whether the nubs can bind to actin.

Visualization of the detailed structure of this region and its relation to the other domains in the head will require X-ray quality crystals of S-1. Selective hinge-cutting enzymes, such as Ps-1, may also prove useful in providing stable intact domains of the myosin head for crystallization. Digestion of native myosin filaments could allow us to examine aspects of the structure of these assemblies which were previously obscured by the size of the myosin heads.

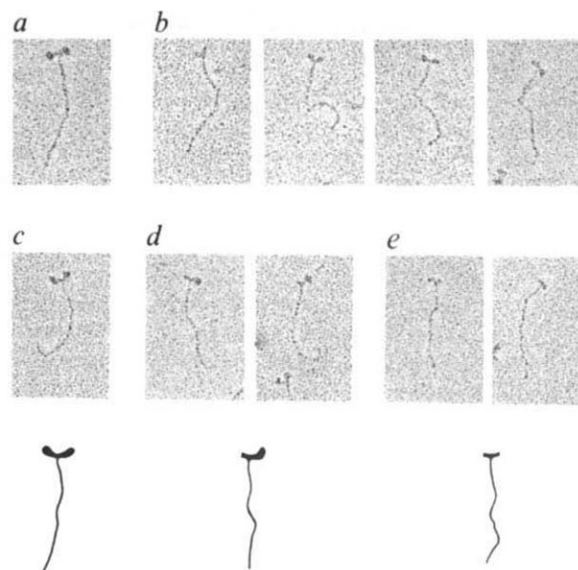


Fig. 4 Electron micrographs of myosin and its Ps-1 rod fragments. *a*, Scallop adductor myosin; *b*, a gallery of scallop Ps-1 rod fragments with associated light chains showing the projecting 100 Å nubs (100 ± 20 Å, *n* = 86); *c*, chicken pectoralis myosin; *d*, chicken Ps-1 rod fragments with one-nub/one-head and *e*, two nubs. The chicken rod fragments were taken from fields of Ps-1 digested myosin in which most of the molecules (>90%) appeared like normal myosin. Tracings of myosin and one-nub/one-head and two-nubbed rod fragments are shown below *c–e*. Replicas were prepared by rotary shadowing with platinum^{14,20}. Molecules were contrasted with ~12 Å platinum coating in *a* and *b* and ~6 Å platinum coating in *c–e*. Magnification ×90,000.

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The elephant and the flea

R.V. Jones

Rutherford: Simple Genius. By David Wilson.

Hodder & Stoughton/MIT Press: 1984. Pp.639. £14.95. (*April publication, \$25.)*

ERNEST Rutherford's towering stature in the heroic age of twentieth-century physics has made him the subject of many books, memoirs and lectures. And yet, in this new and comprehensive biography David Wilson has illuminated fresh facets of the man and his activities.

First there is Rutherford's work in the First World War for the Royal Navy, which has only been sketchily outlined by earlier biographers. In fact, Rutherford was once described as "lacking in public spirit" because, for example, he went on for a visit to his native New Zealand after the British Association meeting in Australia in 1914, while H.G.N. Moseley and H.T. Tizard rushed by ship across the Pacific, by train across the United States, and then by ship again across the Atlantic, so that they could offer their services in Britain (only, alas, for Moseley to be killed at Gallipoli). But David Wilson has discovered in the papers of the late A.B. Wood much about Rutherford's efforts to assist the Navy on his return — from which it incidentally transpires that credit for the invention of Asdic (or Sonar) should be ascribed to Rutherford as much as to his French colleague, Pierre Langevin. And it was not only by invention that Rutherford contributed: he made many visits to establishments and he joined in trials at sea. In 1917 he was pressing the Navy to bring the scientists much more into the operational picture for "in scientific research it was found to be essential that the researcher should have the widest knowledge and personal experience of the difficulties to be solved" — to which a senior naval officer objected "the only information necessary to be given was that enemy submarines were in the sea, and that means were required to detect their presence".

It was to take the experiences of the Second World War, led by the example of Henry Tizard, to convince the Services of the folly of such a view. And Tizard's second contribution, the famous Tizard Mission to the United States in 1940, was by coincidence anticipated in 1917 by a joint Anglo-French Mission led by Rutherford to acquaint American scientists with developments made in Britain and France.

After the war, his campaign for scientific support for the Navy led to the foundation of the Admiralty Research Laboratory.

When, in 1919, Rutherford became Cavendish Professor at the University of Cambridge, his qualities as a scientific administrator were frequently called upon by the British Government, and David Wilson believes that he has made the first study of Rutherford in this capacity. His

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Rutherford (right) at the Cavendish — "spreading tobacco ash and noise, [ruling] the laboratory as a tribal leader".

activities ranged from dealing with individual problems, such as the future of the Artillery College at Woolwich, to being President of the Royal Society and Chairman of the Scientific Advisory Council of the Department of Scientific and Industrial Research, in both of which offices he stimulated much action and prompted many forecasts of national needs; and in 1933 he headed the Academic Assistance Council with the objective of aiding academic refugees from the Nazis.

Wilson has drawn on many sources to depict Rutherford's temperament and character. "Brash and noisy all his life", or more accurately, quoting Henry Tizard:

He had a boisterous sense of fun and a loud laugh. By precept and by example he helped keep our minds free from cant. He hated pomposity and artificiality. He loved simple people and simple ways, and lived a simple life. . . . lived a life of feverish intellectual activity relieved by short periods of magnificent idleness. When he took a holiday it really was a holiday.

Wilson's carefully detailed account of Rutherford's interactions with the many physicists who worked with and around him provides an illuminated cavalcade through forty of the most exciting years in physics: with Soddy, Moseley, Geiger, Marsden, C.G. Darwin, the Braggs, Langevin, C.R.T. Wilson, von Hevesy, Bohr, Chadwick, Blackett, Cockcroft and Kapitza among its members. A whole chapter, in fact, is sympathetically devoted to Kapitza who, Wilson says, came closest to being the son whom Rutherford never had. "The Crocodile" was the nickname given by Kapitza to Rutherford; and this is why, when the Mond Laboratory was built (in Cambridge), Kapitza asked Eric Gill to carve a crocodile over its main door. Unfortunately, the Laboratory had scarcely been opened when Kapitza was irrevocably detained while on a visit to Russia, despite the efforts of Rutherford and many others to persuade the Soviet authorities to release him.

One item which Wilson records has, incidentally, corrected a long standing misjudgment of my own concerning F.A. Lindemann (later Lord Cherwell). When I was in his laboratory in the 1930s I felt that it was unfortunate for physics in Britain that he had not come to an understanding with Rutherford about the division of interests between the Clarendon in Oxford and the Cavendish, instead of the competition exemplified by the 1932 race to liquefy helium and so steal the Mond Laboratory's thunder before it opened. Thanks to Wilson's delving, I now learn that

on his election to the Clarendon in 1919 Lindemann wrote to Rutherford in the following terms: "I am anxious to work in collaboration with Cambridge and the other schools of physics, both to prevent overlapping . . .". Unhappily, relations turned out otherwise.

As background to Rutherford's life and work we are given excellent descriptions of the opening up of New Zealand and of the development of science in Cambridge in the nineteenth century, and in particular of the establishment and achievements of the Cavendish Laboratory in the first seventy years of its existence, occasionally spiced with such episodes as the refusal of the

seventh Duke of Devonshire to allow his sons to have a scientific education despite his own accomplishments of Second Wrangler and Smith's prizeman, and his generous foundation of the Cavendish. Wilson has of course had a great deal of material to draw upon from previous biographies and memoirs, but he has gone through these with a critic's eye and resolved discrepancies between them, and has dug further into the background.

As a result we have a biography which not only exposes Rutherford's contributions in fields which have hardly been treated before, such as his work for the Navy, but also a careful, almost blow-by-blow, account of his researches in Cambridge, Montreal, Manchester and then back again in Cambridge, starting with his magnetic detector for radio waves and his work with J.J. Thomson, leading on to the discoveries which proved him to be the greatest experimental physicist of this century: radon, the alpha-particle, the nuclear atom and nuclear disintegration. Radon, or "emanation", was discovered by the curious observation that the electrical leakage in an air-filled box containing thorium oxide was greater when the door was closed. Both the observation and the remorseless pursuit of its explanation were typical Rutherford.

Just how difficult the pursuit of explanation could be was exemplified by the time it took him to formulate the concept of the nuclear atom after the crucial observation

had been made by Geiger and Marsden, in the spring of 1909, that while nearly all alpha-particles passed through a thin foil as though it were completely transparent, a few were deflected through large angles. "Every schoolboy" ever since has known the explanation: but at the time all the physicists concerned, Rutherford included, were baffled. "It was as though", he said, "you had fired a fifteen-inch shell at a piece of tissue paper and it had bounced back at you." More than eighteen months were to pass before Rutherford could give the news in a letter of December 1910 to a colleague: "I think I can devise an atom much superior to J.J.'s for the explanation of and stoppage of alpha- and beta-particles. . . . It will account for the reflected alpha-particles observed by Geiger. . . ."

David Wilson, a science journalist, is modest about his own command of physics, but I found only one small point where his account might have been helpfully expanded. It concerns Hertz's failure in 1883 to deflect cathode rays electrostatically: when such a superb observer and experimenter failed to find any deflection, the discovery of the electron was probably held up by several years, for it seemed that cathode rays were uncharged. Wilson says that J.J. Thomson suspected that the negative result was due to pressure of gas in the discharge tube, and so repeated the experiment (with the better vacuum obtainable in 1897) when deflection was immediately observed. It was not the residual pressure of gas in the discharge tube which had directly produced the negative result, but the fact that the gas became ionized, and the ions had been attracted to the deflecting plates until they built up a space-charge which neutralized the original deflecting field. The incident is worth remarking for its lesson in showing how one of the greatest of all experimental physicists, with radio waves and the photoelectric effect to his credit, could for once be let down by imperfection of technique.

If I miss anything in this magnificent biography, it is the multitude of anecdotes and apothegms which Rutherford's name immediately recalls. I myself remember, for example, how at a lecture in Oxford in the 1930s, Rutherford squashed E.A. Milne when the latter asked him how he knew that the heavy particles were in the nucleus at the centre of the atom, and not the electrons with the heavy particles orbiting the periphery as in a centrifuge. "Well", boomed Rutherford, who was a big man, over Milne, who was a small one, "when you have an elephant and a flea you assume it is the flea that jumps!"

Those with a taste for more anecdotes can doubtless dig for them in the seventeen pages of notes and five pages of bibliography with which David Wilson concludes his account. It is simply one of the best. □

R.V. Jones is Emeritus Professor of Natural Philosophy at the University of Aberdeen.

One objective for physicists

Alan H. Guth

Unity of Forces in the Universe.

By A. Zee.

World Scientific (distributed by Wiley):

1983. Two volumes, pp.1,076. Each volume hbk £40, \$57; pbk £16.50, \$25.

To a particle physicist, the 1960s now look like the Dark Ages. Of the four known physical interactions, electromagnetism was the only one for which a totally successful theory existed. However, the last years of the decade marked the beginning of a period of enormous activity. Not since the 1920s had physicists made such rapid and exciting progress in understanding the fundamental laws of nature. The work of Weinberg, Salam, Glashow and 't Hooft was synthesized into a perfectly acceptable theory of the weak and electromagnetic interactions. Meanwhile the theory of quantum chromodynamics emerged, and it gradually came to be regarded as the correct theory of the strong interactions. Thus, by the early 1970s, three of the four interactions were understood, with only gravity (at the quantum level) remaining a deep mystery.

With morale bolstered by these triumphs, particle theorists then began an attempt to unravel the physics of energy scales far beyond those that can be reached in available or even foreseeable accelerators. The watchword of this new movement in particle theory is *unity*. Just as physicists of the nineteenth century discovered that electricity and magnetism are inextricably linked, their twentieth-century heirs are now trying to show that *all* the interactions in nature are simply diverse manifestations of a single unifying force.

The movement towards unity in physics has given rise to a vast number of published papers. For example, there are now over 1,300 which cite the original work of H. Georgi and S. Glashow on the SU(5) grand unified theory. In preparing his two-volume collection, A. Zee has carefully chosen 78 papers to illustrate the important developments in the quest for unity. The papers cover many aspects of the general theme, and thereby reflect admirably the exciting developments in particle theory over the past decade. In addition, Zee has provided about 100 pages of lucid summary and explanation to serve as a companion and guide to the reprinted papers. The reader is assumed to be familiar with the basics of quantum field theory, including non-Abelian gauge theories. For prospective readers who lack this background, the author has included a short list of suggested sources.

The two-volume set is divided into 18 chapters, each of which contains a few pages of explanatory text and several

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reprinted articles. The explanations are concise yet clear, summarizing the important developments and unsolved problems in a given area. Although the information is densely packed, the style maintains a flavour of informality. With liberal use of quotation marks, Zee writes about the "see-saw mechanism" of generating neutrino masses, and of the "lopsided" versus the "orthodox" pattern of symmetry breaking in the $SO(10)$ grand unified theory. He also informs us, in a footnote attributed to Howard Georgi, that the $SO(10)$ theory was actually discovered half an hour before the $SU(5)$ theory. I am still trying to imagine how breathtaking a day that must have been.

Volume I is concerned mainly with the basics of grand unification, founded on the groups $SU(5)$, $SO(10)$ and $E(6)$. This volume also includes a two-part "Instant Review of Group Theory", as well as chapters on proton decay, and on attempts to understand the existence of three families of fermions and to determine the fermion masses. Volume II begins with five chapters on cosmology, emphasizing the impact of recent developments in particle physics. Writing primarily for particle physicists, Zee begins with a short course on basic cosmology. The explanatory notes will be particularly useful to the beginner,

and the reprints include many of the classic papers of George Gamow and his collaborators. Subsequent chapters deal with the generation of the matter-antimatter imbalance, the theory of galaxy formation (with or without massive neutrinos) and the inflationary universe model. The remainder of Volume II includes accounts of dynamical symmetry breaking and composite models, as well as a brief discussion of the attempts to include gravity in the programme of unification.

A quality of the books which I particularly admire is the author's frank and unassuming tone. Zee points out that "the selection [of papers] assuredly reflects the author's idiosyncracies and the fact that while he has certainly not read every paper in the field, he has definitely read his own papers". (In fact, the books contain only six of Zee's articles, all of which seem quite appropriate to me.) In an effort to encourage the student who might be frightened by the vast volume of literature in this field, Zee points out that "it is not even necessary to read all the papers reprinted in this volume. This writer certainly hasn't". Nor, I confess, have I. □

Alan H. Guth is an Associate Professor of Physics at the Massachusetts Institute of Technology.

Basic methods

Roger Patient

Techniques in Molecular Biology.

Edited by John M. Walker and Wim Gaastra.

Croom Helm/Macmillan, New York: 1983. Pp.333. Hbk £19.95, \$29.95; pbk £9.95.

THE success of a textbook, like that of a lecture, is dependent upon a clear idea of the likely audience: the authors and editors of *Techniques in Molecular Biology* appear to have largely satisfied this important criterion. The main readers will be final-year undergraduates (especially project students) and postgraduates embarking on research. Established research workers will find most of the book familiar, but will value its presence in the laboratory when employing a new technique or when suffering one of those irritating failures of a technique which has been working for years. In addition useful tables and titbits of information are sprinkled throughout.

The scope of the book is fairly comprehensive, covering the separation and *in vitro* synthesis of proteins and nucleic acids, their transfer to solid supports and detection, determination of their amino acid or nucleotide sequences, and molecular cloning. Very little is considered too basic for inclusion: nick translation, for instance, gets a whole chapter. The information is well-organized and concisely

presented, with a lot of internal cross-references. In relation to other literature available, this publication intercalates quite nicely between *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, 1982), which contains an enormous amount of practical detail, and the *Genetic Engineering* series, published by Academic Press, which contains none.

Criticisms of the book centre on a very inconvenient way of listing references and a number of omissions or incomplete treatments of particular techniques. For instance, there is no good description of the direct application of nucleic acids to nitrocellulose and subsequent hybridization (the so-called "dot-blot" technique), and only cursory treatment of *in situ* immunoassay techniques for screening bacterial recombinants. Occasionally, the information provided is actually misleading, as in the statement that ribonuclease-free deoxyribonuclease is available commercially (in practice, further purification is usually necessary before use). Finally, the problems associated with particular techniques are sometimes glossed over, as in the cases of M13 nucleotide sequencing, which has taken a number of laboratories a long time to establish, and assays of mRNA solutions using 3H polyuridylic acid, where problems with variability of polyadenylic acid tail length and reproducibility are often experienced. □

Roger Patient is a Lecturer in the Department of Biophysics at King's College, University of London.

Bangosomes: the past and the present

J.A. Lucy

Liposome Letters.

Edited by A.D. Bangham.

Academic: 1983. Pp.421.

£17.50, \$29.

Liposomes.

Edited by Marc J. Ostro.

Marcel Dekker: 1983. Pp.400.

SwFr.157, \$58.75.

Liposome Letters is a most unusual if not unique book, but that will not surprise those who are personally acquainted with the editor. In 1982 Alec Bangham wrote to some fifty scientists who have been concerned in one way or another with liposomes to suggest they send him informal letters, poems etc., that chronicle an anecdote, observation, hypothesis or comment about liposomes. His intention was that their offerings should recapitulate the developments and diversification of the liposome "membrane" model from its early days up to the present. In the preface he says that this formulation derived from a feeling that too much of our scientific writing is stereotyped, banal, platitudinous and devoid of all sensibility to the fabric of life in scientific research, success or failure. After just twenty years, I now realize why Alec considered my initial draft of a joint paper with him to be "pretty turgid stuff".

It is surprising what one can find in this volume. For example I see that in the preface by Papahadjopoulos, I and three other named villains are pilloried for apparently considering that he and Bangham were simply wasting their time in the early stages of work on liposomes. Then, Racker's chapter is punctuated by five Lessons, the last of which reads:

After you publish rejoice when other scientists don't believe what you know to be true. It will give you extra time to work on the phenomenon in peace. When they start claiming that they have discovered it before you, look for a new project.

His chapter ends with full-page photocopies of two letters, one from Racker to Bangham that reads "The answer to your question in your letter of March 1, 1982, is yes", and the other from Bangham to Racker replies "Thanks". Scherphof has contributed a witty three-page dissertation on how to pronounce the word liposome that includes a "Light-micrograph of the essential part of a Dutch technician caught in the act of preparing [blowing] an animal-size hemilamellar liposome (ASH-L). Bar is 1,0cm". The text says "For anybody watching this picture there cannot remain a shadow of doubt that a liposome is a LIP'əsōm', can there? From Groningen, with LUV, P.S. You should see her in full colour!".

Most papers are, however, primarily serious but with lighter touches, such as the Molecular Trojan Horse that illustrates the article by Dimitriadis on the transformation of eukaryotic cells, and perhaps most of the contributions would not seem particularly out of place in a more conventional book. (Each contribution has been photographically reproduced from the original typescript, but none is difficult to read.) There are some 40 articles in all, from which much can be learned about liposome lore. Anyone who has been personally involved with liposomes, or who knows workers in the field, will also be interested by the anecdotes. Those who have not might find them slightly tedious. The unusual format has, however, encouraged authors to be more forthright than usual, and this is a valuable feature in itself. For example, to quote finally from George Poste:

I was highly skeptical (and remain so) about the feasibility of targeting liposomes to tumor cells in solid neoplasms and their metastases *in vivo*. . . . My criticisms are directed only to those proponents of targeting who chose to ignore the substantial problems created by anatomic and physiologic factors *in vivo*. . . . Overselling always carries the danger that real opportunities may be lost because of skepticism elicited by earlier extravagant claims which have failed to come to fruition.

Although *Liposomes* edited by Ostro is of comparable size to *Liposome Letters*, and was published at about the same time, it appears to have taken longer to produce perhaps because it is not based on camera-ready copy. There are eight contributions, five of which are from the pens of authors who also appear in Bangham's volume. In Ostro's book, however, their contributions are basically more serious (influenced by Bangham, I nearly wrote more prosaic), or rather all of them except Bangham's own short historically-orientated chapter. The editor has attempted to assemble reviews of various areas of liposome research in such a way that the volume represents a compilation of the major work in the field since 1965 (up to, as far as I can see, fairly early in 1982).

In the preface Ostro (whose own affiliation is The Liposome Company) comments that 30% of those attending a recent meeting on liposomes were affiliated to commercial laboratories. This is a useful laboratory reference book and it similarly reflects current interest in the practical and commercial applications of the targeting of liposomes as delivery systems. Thus the three chapters (liposomes as a tool in molecular biology; immunologic aspects of liposomes; therapeutic applications of liposomes), which are most concerned with various aspects of this topic, occupy considerably more than half of the volume. □

J.A. Lucy is Professor of Biochemistry at the Royal Free Hospital School of Medicine, London.

Catastrophe, chaos and delay

Athel Cornish-Bowden

Mathematical Models in Molecular and Cellular Biology.

Edited by Lee A. Segel.
Cambridge University Press: 1983.
Pp. 768. Pbk £15, \$29.95.

WHY is the story of the mathematician who opened a lecture with the words "Consider a spherical elephant" not funny? Biologists need to understand this before they can appreciate the value of mathematics and overcome their suspicion of it. Some caution is justified, as there are many who seem to regard numbers and equations as a convenient shield for the poverty of their ideas, but mathematics has made considerable contributions to biology and deserves to be taken seriously.

To give one example: a valid theory of metabolic control cannot be developed without a mathematical framework that extends beyond the elementary kinetics of isolated enzymes; in consequence, biochemists who reject a mathematical approach continue to be misled by oversimplified ideas, such as "bottleneck enzymes", "controlling steps" and so on. In some areas of biology it is easy to check by experiment whether a mathematical theory makes correct predictions, even if one does not understand the mathematics. Metabolic control, however, is difficult to study by experiment (other than by computer simulation), and so one cannot easily convince the sceptic that metabolic fluxes must be largely unaffected by moderate changes in the concentration of almost any enzyme. In such cases scientists who deny themselves all but the most elementary mathematics are also denying themselves understanding.

Anyone genuinely interested in learning how mathematics is being applied to research problems in biology can hardly do better than begin with *Mathematical Models in Molecular and Cellular Biology*, a book first published in 1980 and now available in paperback. Dr Segel has taken his responsibilities as editor seriously and has produced a real book, not just a haphazard collection of papers. Indeed, the individual authors are given so little prominence that their names do not appear at the beginnings of their chapters.

I particularly liked the obvious efforts taken to support the mathematics with a readable text and to make it believable. For example, the information that the equation $x_{t+1} = rx_t(1-x_t)$ can generate chaotic (aperiodic) behaviour is accompanied by the instructive suggestion that "the reader simulate the difference equation on a hand calculator to see this". After doing just that, I found it much easier to accept the important biological message that

"phenomena that look chaotic may come from simple underlying laws". As the author of that chapter remarks, "the implications . . . are most unsettling"!

I was disappointed to see that population genetics is mentioned only in passing, but this was the only major omission that I noted. Some other topics, such as elementary thermodynamics and enzyme kinetics, are also dealt with briefly or not at all, but there would be little point in filling a book of this kind with the staples of general biochemistry texts.

Catastrophe, chaos, delay. . . . It will be evident by now that the title of this review is not a comment on the book: it is inspired by the editor's list of some of the mathematical concepts to be discussed in a biological context, but there is much else to interest anyone with a lively mind. □

Athel Cornish-Bowden is a Lecturer in the Department of Biochemistry at the University of Birmingham, UK, and the author of *Basic Mathematics for Biochemists* (Chapman & Hall, 1981).

Sex in society

Stuart Sutherland

Men and Women: How Different Are They?

By John Nicholson.
Oxford University Press: 1984. Pp. 193.
Hbk £9.95, \$19.95; pbk £2.50.

ONE OF the more curious aspects of social research is that it so rarely produces definitive results. This is especially true in the area of sexual differences and their origins: *Men and Women* is a rather cursory survey of the evidence on this topic. It is intended as a popular book and rarely cites primary sources or gives enough details of experiments and surveys to allow the reader to judge for himself — or, one should perhaps add in this context, herself. John Nicholson appears to be bent on showing first that sex differences are either non-existent or small, and second that where they exist they are caused by socialization not by genetic effects. His arguments are often cunning, but that does not prevent many of them from being fallacious. Here are some examples.

In attempting to disprove the existence of an innate maternal drive, he uses as a counter-argument baby-battering by mothers. This is like arguing that hunger is not largely innate because some people are anorexic. Again, he argues that even in animals the maternal drive is a weak one, because it often disappears if the mother is separated from her offspring for a short time. But the development and maintenance of many innate drives are known to depend on the presence of the appropriate stimulus. Given the importance of maternal behaviour for the survival of the species, it would be remarkable if no genetic

provision had been made for the appropriate behaviour along with the appropriate physical apparatus for nurturance.

Nicholson's treatment of differences in aggression is equally odd. Having quoted a mass of evidence that suggests that men are more aggressive than women, he writes "In the absence of evidence to the contrary, I suggest we assume that men *are* more aggressive than women". Elsewhere he tries to dispose of the argument that the greater tendency of males to be aggressive is — at least in part — caused by the presence of higher levels of testosterone. He cites the case of girls who were exposed as fetuses to exceptionally high levels of androgens and notes that their behaviour tends to be tomboyish, but he fails to draw the obvious conclusion.

Despite his bias against biological explanations, Nicholson writes clearly though without much wit or charisma. Some of the results he provides may titivate. Half of American fathers have never changed a nappy — not even a disposable one; the difference in the athletic records of men and women has been enormously reduced over the past 50 years (one hopes this has nothing to do with steroids); women's scores on examinations are not influenced by the point in the menstrual cycle at which they take them; men and women do not differ in self-esteem, ambition or ability, but men are the more confident sex.

Since Nicholson virtually rules out innate factors as responsible for the differences between men and women, he is not unnaturally puzzled that all known societies are patriarchal: men occupy the prestigious jobs, and in encounters between members of the opposite sex, both men and women almost invariably feel that the man is dominant. One of his proposed solutions is that men's aggression and desire to dominate stems from their shame that they cannot have babies, a proposal that could surely be disposed of fairly quickly by a social survey — unless of course the hurt goes so deep that men are ashamed to admit it. There is another possibility that he does not consider. Perhaps men and women are innately disposed to act in different ways to members of the opposite sex. This would account for much of the evidence he quotes, for example, the fact that both men and women treat boy and girl babies differently even when they have been misled about the sex of the baby. The initial bias would determine the process of socialization, which would in turn increase the bias. The explanation would also account for one of Nicholson's recurrent themes: despite the feminist movement, it is hard to demonstrate any significant changes over the past 20 years in attitudes or practices relating to the interaction of the sexes. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition, University of Sussex.

Histochemistry and cytology

Robert Olby

History of Staining, 3rd Edn.

By George Clark and Frederick H. Kasten.

Williams & Wilkins: 1983. Pp.304.

\$29.95, £22.25.

A SERIES of articles published in the journal *Stain Technology* between 1928 and 1933 formed the text of the first edition of this book, which was compiled by H. J. Conn and appeared in 1933. With the additions to the second (1948) and present (third) editions, the book is now virtually twice its original size. Successive editions have followed Conn's intention, which was to cover the field broadly rather than going deeply into specialized topics. As is typical of collections of reprinted papers, there was in the original book a lack of any overall thesis which might have acted as an organizing principle; unhappily, this feature has been perpetuated rather than overcome in the later editions.

The subject matter in this new edition has been organized in terms of, first, the different sorts of dyes, second, the several kinds of biological materials — bacteria, blood, connective tissue, nerve tissue, antibody and antigen — and, finally, selected major figures such as Sir John Hill, Joseph von Gerlach, Rudolf Heidenhain, Paul Mayer, Paul Unna, Paul Ehrlich, Walther Flemming, John Belling, Gustav Mann, Frank Mallory and Ralph Lillie. It is not clear why Rudolf Heidenhain was chosen rather than his son Martin, author of the iron-haematoxylin staining procedure and pioneer in the use of aniline dyes, but the other figures are reasonable choices.

The early history of biological staining centres on the use of carmine from the cochineal insect and haematoxylin from extract of logwood. Next came the aniline dyes, following upon Perkin's production of "mauve" in 1856 — safranin, methyl violet, aniline blue, eosin, methyl green, methylene blue, acid fuchsin and so on. The golden era of cytology in the 1880s and 1890s, in which the details of the chromosomes, and the nature of cell division and fertilization were revealed, rested upon the growing range of stains and techniques described in the book.

The history of the relationship between histochemistry and cytology is a fascinating one which goes back to the very early days. In his chapter on protein and nucleic acid histochemistry, Professor Kasten takes us back to François-Vincent Raspail's use of the starch-iodine reaction in 1825. He stresses the importance of Miescher's and Zacharius's study of the relation between nuclear staining and the chemistry of the nucleus. But it was

Feulgen's "nuclear reaction" which Kasten sees as the decisive influence in establishing the localization of proteins and nucleic acids in the cell, paralleling the distinctive staining reactions of nucleus and cytoplasm, and overturning the old belief in the restriction of DNA to animal tissues and RNA to those of plants.

There are obvious difficulties in writing about a collection of techniques and leading technicians for anyone but specialists. No doubt this text will be much appreciated by them. The general reader, however, will surely require a very different sort of book, one organized not around specific stains and famous figures but concentrating on general issues, such as the much-debated question of the existence of organized structures in the cell, their persistence and their replication. Intimately connected with this issue were fundamental questions over the nature of fixing and staining themselves. Did the process of fixing create structures which then adsorbed the stains to produce a spurious architecture within the cell? Was staining simply physical adsorption as Albert Fischer maintained? Were the cytoplasmic organelles discovered in the nineteenth century mere artefacts as W. B. Hardy held? This debate is mentioned on p.199 but not developed. From the book, one would not have guessed that the tenets of cytology were seriously undermined by these fundamental criticisms, and that the emerging tradition of experimental cytology, its practitioners having become sceptical of the claims of classical cytology, concentrated upon the biophysical properties of protoplasm and the presence of surface phenomena in the cytoplasm.

Needham and Peters in the 1930s envisaged a "cytoskeleton" in the cytoplasm which was a colloidal formation and not an organelle in the sense of classical cytology. This rejection of the tradition of classical cytology was not reversed until the techniques of freeze-drying, electron microscopy and ultracentrifugation had been introduced in the 1930s and successfully developed in the 1940s. Kasten points out how important was Feulgen's "nuclear reaction" in this change of opinion. It should also be stressed that histochemists were in the van of the movement beginning in 1950 to establish nucleic acids rather than proteins as the hereditary material. This decision arose from the results of the quantitative study by histochemists of the content of DNA and protein in the chromosome cycle of dividing cells and from the discovery of the halving of DNA content during reduction division. That the contribution of the histochemists is often ignored suggests that this tradition has yet to receive a just evaluation from historians. The present book offers an introduction and valuable bibliography for any such attempt. □

Robert Olby is Reader in the History of Science at the University of Leeds.

Scottish geology for the 1980s

W.S. McKerrow

Geology of Scotland, 2nd Edn.

Edited by G.Y. Craig.

Scottish Academic Press/Halsted: 1983.

Pp.472. Hbk £35, \$54.95;

pbk £17.50.

Scotland's Environment During the Last 30,000 Years.

By Robert J. Price.

Scottish Academic Press/Columbia University Press: 1983.

Pp.224. Hbk £27.50, \$41.50; pbk £15.

GEOLOGICALLY, Scotland is a small but significant part of the world. Its standing is due to a combination of factors: diverse geology, good exposures and attractive landscape for the field geologist, and especially its wealth of geological literature. James Nicol was very wrong when he stated (in his 1844 *Guide to the Geology of Scotland*) that "...in a country which has been so frequently investigated by eminent geologists, it is evident that little absolutely new can be expected". The reverse is true: the better documented the geology, the easier it is to make further advances. Scottish studies in deformation, metamorphism, igneous petrogenesis and Palaeozoic stratigraphy have all led the world at different times during the past two centuries. The names of Hutton, Judd, Geikie, Lapworth, Peach and Horne, Barrow, Harker, Bailey, Richey, Read and Kennedy help to remind us of some of the advances which came from Scotland. What is Scotland doing for geology today?

Since 1965, when Craig produced the first edition of *Geology of Scotland*, our understanding of geological processes has progressed greatly. We now know more about the rates of crustal processes, structural lineaments, the deep crust, palaeolatitudes, heat flow and the continental shelf around Scotland. We also have plate tectonic models to assist us in finding analogues (from other times and other continents) to some of the major tectonic events in the geological history of the area. Most of these advances are recorded in the new edition, but the different contributors each have their own personal approach to the new facts and the new ideas. We now have much clearer views on the significance of many rocks and structures which previously were baffling or just interpreted wrongly.

Of the 12 authors, seven contributed to the first edition and have attained somewhat variable success in bringing their essays up to date. All five new contributors have maintained the high standards set in the first edition. In his introductory chapter, Harris gives a clear outline of Scottish geological development, much enhanced by his table of events (Fig. 1.2). It

is unfortunate that the sections on Tertiary growth and on geomorphic evolution (present in the first edition) have disappeared; these are relatively neglected fields that should not be ignored.

The presentation and standard of editing is, in general, so good that it is perhaps unnecessary to stress the few failures. A stronger hand might have removed some hangovers from the first edition, such as the multiple discussions of the Highland Border Group (presented separately on pp. 84, 98 and 109–110) and of the Ballantrae Volcanic Group (pp. 110, 152 and 174–175). The editor might also have established some standardization of such a crucial term as "Caledonian Orogeny", where Scottish geologists should give a clear lead. Now we know that many orogenies extend over long periods of time (few are short-lived as Stille thought), we badly need to get our definitions sorted out.

One achievement of the book is that diverse parts of geology have been brought together to form a coherent unity, although this does break down in a few places. The new contributions by Brown and Mykura respectively provide excellent summaries of Devonian magmatism and stratigraphy, as do the chapters by Francis on the Carboniferous and by Lovell on the Permian and Triassic, but it is a pity that these accounts of the Late Palaeozoic igneous rocks and sediments have not been more closely integrated.

The contribution by Emeleus on Tertiary igneous rocks contains clear reviews of the latest work in geochemistry relevant to petrogenesis. This chapter takes cognizance of recent geophysical studies, though in general geophysics is not well discussed throughout the book — such a failing is to be regretted, especially in view of much recent work on the deep geology of Scotland.

Sissons gives a brief summary of the complex developments in Scotland during the Quaternary. His ability to condense a large amount of data becomes evident by study of another new contribution to Scottish geology, *Scotland's Environment During the Last 30,000 Years*. The author, R.J. Price, states (p.23) that:

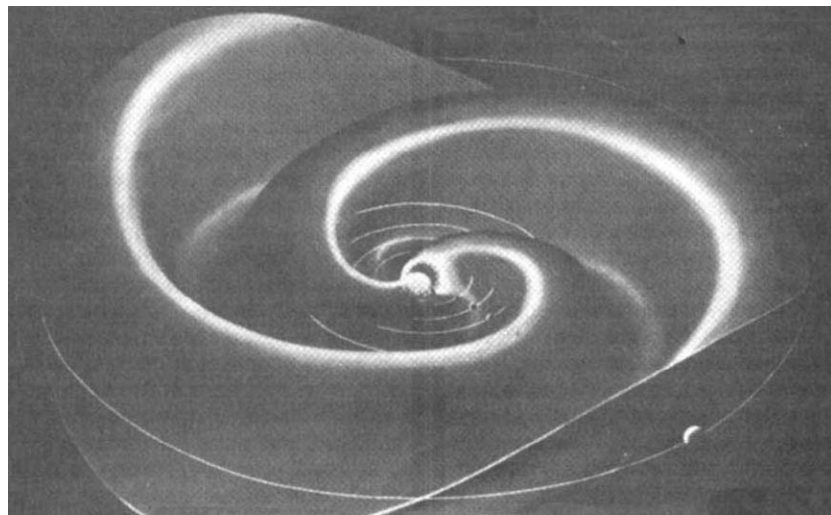
This book is concerned not only with the geomorphological and marine sedimentary record but with the broad environmental changes which can be identified as a result of detailed investigations not only in these fields but in all aspects of the geomorphological, stratigraphical, palaeontological and archaeological record.

Price lives up to his claim in discussions of geomorphology and chronology, but some geologists will find him weak on sedimentology.

Price emphasizes a most important geological truth (pp. 8–11): the record is best in the oceans, where sedimentation has been more or less continuous; it is next best on the shelf, then on coasts and low ground, but it is almost non-existent in mountains. As a result, the key to many recent advances in Quaternary stratigraphy lies on the sea floor. Will river terraces be the next sediments to repay renewed investigation? They have produced many good results in the English Midlands; could they not also do the same in Scotland?

Both books reviewed here are clearly presented and well illustrated, with full lists of references. They should prove useful to a wide readership: the scientist, the student and the scientifically orientated tourist. Scotland has still further significant contributions to make to geology, and these two books will help to provide a sound basis for future work. □

W.S. McKerrow is a Lecturer in the Department of Geology and Mineralogy at the University of Oxford, and a Fellow of Wolfson College.



An artist's conception of the interplanetary medium out to the orbit of Jupiter, showing the spiral structure of the interplanetary magnetic field and the wavy shape of the interplanetary neutral sheet. (The neutral sheet, which is invisible, divides magnetic field lines pointing towards the Sun from those pointing away.) The illustration is taken from *Astronomy from Space: Sputnik to Space Telescope*, edited by James Cornell and Paul Gorenstein, and recently published by MIT Press. Price is \$17.50, £15.75.

J. Wilcox, Stanford University

Equipment for microbiology

This week's selection of new products covers all those that would be used in a microbiology laboratory — from reagents to immunoassay kits.

Biologicals

● Endothelial cell growth supplement (ECGS) is now being supplied by Stratech Scientific. ECGS has been shown to replace feeder cell layers in clonal growth of hybridoma and other cell types. It shows consistency in growth-promoting activity, simplifies culture maintenance and is free from detectable bacteria, fungi and mycoplasma. It is supplied in a lyophilized form which is stable for 2 yr at 4°C. One vial, containing 15 mg, will supplement up to 300 ml of medium.

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● Advanced Laboratory Techniques has now added a number of fluorescein (FTC) labelled compounds to its reagent range. These compounds are immunoreactive and intended for use in immunoassay systems. Included in the FTC-labelled range are: amikacin, carbamazepine, digoxin, human placental lactogen, phenobarbital, phenytoin, L-thyroxine and tobramycin. Most of these compounds may be conjugated to various alternative fluorescent labels.

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● Amersham has added six new restriction endonucleases and one new modifying enzyme to its range of restriction and modifying enzymes. The additions are: *Acc II* (isolated from *Acinetobacter calcoaceticus*); *Ava I* (isolated from the blue green alga *Anabaena variabilis*); *Ava II* (isolated from the blue green alga *Anabaena variabilis*); *Dra I* (isolated from *Deinococcus radiophilus*); *Eco RV* (isolated from *Escherichia coli* J62 pLG74) and *Sac I* (isolated from *Streptomyces achromogenes*). The modifying enzyme is DNA polymerase I from *E. coli*.

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Anglian Biotechnology has asked us to make an addition to our report on avian myeloblastosis virus (AMV) reverse transcriptase (see *Nature* 307, 196). They would like us to point out that the product is manufactured using a technique pioneered by the president of Molecular Genetic Resources, Dr G.E. Houts, and that any queries involving AMV reverse transcriptase should be directed to Molecular Genetic Resources Inc., 6201 Johns Road, Suite 8, Tampa, Florida 33614, USA.

● A range of specific protein rich media is now available from Advanced Laboratory Techniques. The media are suitable for the preparation of serum standards for immunoassay or as a source material for the preparation of protein. The following are immediately available: C-reactive protein (CRP), liver ferritin, placental lactogen (PL) and thyroxine-binding globulin (TBG). All the media are supplied freeze-dried and sold as weight of specific protein. Small samples are available for evaluation.

Circle No. 103 on Reader Service Card.

● Two new mounting media, hydramount and dimethyl hydantoin formaldehyde resin, have been added to the BDH Gurr range of stains and microscopy materials. Hydramount is a fast-drying aqueous mounting medium which has adhesive properties when set so that the problems of specimen damage resulting from movements of the coverslip are minimized. Hydramount is suitable for botanical and zoological applications. It has a refractive index of 1.4668 and a viscosity of 4.71 cP at 20°C. It is designed for use in micro-entomology or in the preparation of aquatic micro-invertebrates. Dimethyl hydantoin formaldehyde resin (DMHF) is a water-soluble resin for mounting whole or dissected entomological material. It is designed for use in preparing taxonomic specimens for reference collections.

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● An improved version of the microbore valve has been introduced by Rheodyne. The new model features 0.2, 0.5 and 1 µl chambers, a 0.13 mm diameter outlet passage and a built-in syringe needle part with 0.3 µl hold-up volume. The previous microbore valves (7413, 7410) are still available, but the 7520 is designed to replace them in areas of microbore HPLC where sample size is critical.

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● Colony stimulating factor and human interleukin 2 are now being produced by Genzyme Koch-Light. Colony stimulating factor (mouse), colony stimulating factor (human) and interleukin 2 (human) can all be supplied. The new lymphokines add to Genzyme's existing range of research biologicals — interleukin 1 (mouse), nerve growth factor (human) and collagen II (chicken).

Circle No. 106 on Reader Service Card.

● The new hand-held UVX Radiometer from UVP has three interchangeable UV sensors. Each sensor has a 36-inch electrically-shielded cable enabling accurate readings from inaccessible locations. Designed for general use within UV-A, UV-B and UV-C wave bands, the sensors come equipped with filters to reduce the solarization characteristically associated with 254 nm UV sensors. The UVX Radiometer features direct digital readout, automatic test circuitry and a read-hold switch.

Circle No. 107 on Reader Service Card.

● RNasin is now available from Anglian Biotechnology. RNasin is purified by Promega Biotec and can be used in *in vitro* translation systems using rabbit reticulocyte lysate and in cDNA work using avian myeloblastosis virus reverse transcriptase.

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● Two new endoglycosidases are available from Boehringer Mannheim Biochemicals for use in structural studies of glycopeptides and in the removal of sugars from glycoproteins. These glycosidases show very different specificities although both should be used in studies where specific cleavage of glycopeptides or glycoproteins is desired. Endoglycosidase D is specific for the digestion of complex sugars, while endoglycosidase H is useful for the digestion of high-mannose chains.

Circle No. 109 on Reader Service Card.

● Beckman has announced an addition to its range of Ready-Solv scintillation counting cocktails. The new CP extended clear-phase cocktails solubilize aqueous samples for counting. The cocktails hold up to 33% of most aqueous samples as a continuous clear liquid emulsion from neat to maximum capacity with no two-phase breaks. It will count the standard sample size of 1 ml in miniature counting vials. It can also be used for the elution of samples in filter counting techniques and for high protein content samples. Ready-Solv CP can be used in any liquid scintillation counter.

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These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

General laboratory equipment

● The MSJ-T series of fermentors from Bioengineering Associates automates the production of large batches of plant or animal cell cultures. The fermentors are designed for use with cell suspensions or microcarrier beads. Models are available in vessel sizes of 10, 20, 30, 40, 50, 70 and 90 litres. The system is controlled by the microprocessor-based Bioprocessor which automates control of pH, dissolved oxygen and temperature. It also runs steam sterilization of the vessel and the medium. Twelve analog and eight digital input ports are available. A computer can be added via the RS232 serial port or the optional IEEE-488 interface bus. In addition to the MSJ series, Bioengineering Associates can supply the MD series of bench top fermentors which are offered in five vessel sizes from 1.2 to 10 litres.

Circle No. 111 on Reader Service Card.

● Four new thermostatic water baths are available from PolyScience. The units feature a durable polypropylene tray equipped with a heater, circulating pump and a temperature control circuit. The operating range is from ambient to 50°C with a control accuracy of $\pm 0.1^\circ\text{C}$. These baths are useful in chemistry, biology and photographic laboratories as well as blood bank applications.

Circle No. 112 on Reader Service Card.

● AMS (Analytical Measuring Systems) has announced the release of a new image analyser to augment its existing system III and VIDS ranges. Called 40-10, the instrument is specifically designed for routine laboratory use. Applications include fluorescent bacteria counting, colony counting, area fraction measurements, diffusion zone area measurements, porosity and cell counting. The system is microprocessor-controlled and includes a built-in printer. The detector is digital, giving 256 grey level settings, and there is an automatic detection system.

Circle No. 113 on Reader Service Card.

● Hybritech has released the Gamma-Photon system for isotopic immunoassays. The solid state 24-well counter allows sample throughput of 500 tubes per hour, and provides for automated data reduction using a Hewlett-Packard model 87XM computer. The instrument offers data reduction software which gives data reduction of Hybritech's immission rate measuring apparatus format solid-phase monoclonal antibody-based Tandem-R immunometric assays. Gamma-Photon also includes data reduction routines for conventional competitive-binding radio-immunoassays, and for screening assays including hepatitis tests and pregnancy tests.

Circle No. 114 on Reader Service Card.

● The Titer-Pak from Robbins Scientific consists of a plastic box with telescoping cover. This contains a removable 96-hole rack which is pre-loaded with pipette tips. It is designed for use with multi-nozzle and standard pipettors. The complete Titer-Pak is disposable and is made of autoclavable polypropylene. It is also available sterilized by radiation.

Circle No. 115 on Reader Service Card.

● A three-dimensional hydraulic microdrive has been introduced by Medical Systems. The MO-103 is small and features a driving actuator which is remotely located from the headstage. Axes x, y and z can be driven smoothly through a cubic centimeter without zigzagging or limping. Control knobs are graduated in steps of 2 μm .

Circle No. 116 on Reader Service Card.

● The new Harris Lo Temp 16-V upright freezer has a capacity of 16 cubic feet divided into five chambers. Each chamber is fitted with an insulated, magnetically-sealed sub-door. The freezer is designed for life sciences, pharmaceutical and blood applications and comes in three models: -40, -70, or -85°C low temperature point. Standard on all models are casters and a safety handle and options include air or auxiliary water cooling and a standby CO₂ system.

Circle No. 117 on Reader Service Card.

● Heraeus has introduced three new types of tissue culture dish to the British market. Flexiperm is a reusable culture vessel made of silicon rubber. Its lower surface adheres to all smooth surfaces to form containers for cell culture. Two containers are available: Flexiperm slide which is designed for use with 76 x 26 mm microscope slides and Flexiperm disk which is normally used in conjunction with a Petriperm culture dish. The Quadriperm is a rectangular cell culture dish with four subdivisions in which cells may be cultivated on standard 76 x 25 mm microscope slides.

Circle No. 118 on Reader Service Card.

● The Mini Reader II is a manual ELISA reader produced by Dynatech which is coupled to a printer. It incorporates a plug-in cartridge programming system. A 96-well micro-ELISA plate can be read by the instrument and the results printed in approximately 2 min. The machine can store a series of readings and will print these readings when required in either a tabular format, a matrix showing positive and negative values or in optical density readings. In addition, the Mini Reader II provides a visual display of readings, well-by-well, with the results shown on the LED display. The machine has an integral keyboard for data entry which permits the user to select blanking on any well of the plate or number of wells in addition to permitting other data entry requirements, such as stored information on print on demand used in conjunction with the program cartridges.

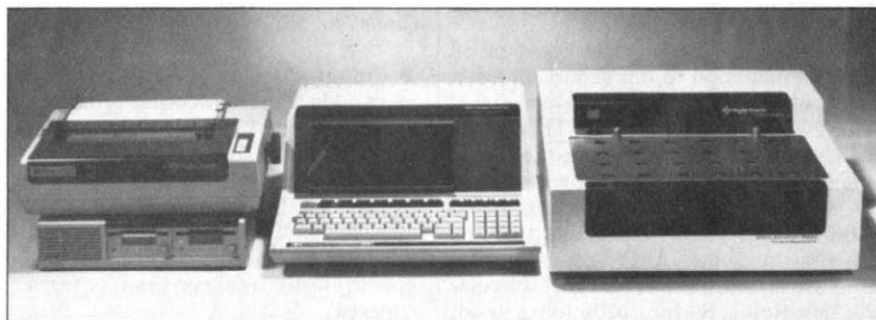
Circle No. 119 on Reader Service Card.



The image analyser from AMS



Dynatech's Mini Reader II



The GammaPhoton system from Hybritech for isotopic immunoassays



Elkay's pump tubing for Astra

● With the introduction of new Elkay pump tubing for use with Beckman Astra analysers, there is now an alternative single source for plastic consumables for Astra instruments. The silicone tubing is packaged in a press snap lidded polystyrene box.

Circle No. 120 on Reader Service Card.

● LDC/Milton Roy has released two new versions of the spectroMonitor, model 1204A, variable wavelength detector. One version includes a maxN series fluid cell and a digital absorbance display. The standard version of the spectroMonitor III detector includes a 1,000 p.s.i. high pressure fluid cell and an analog absorbance display. The spectroMonitor III baseline drift is 5×10^{-4} A h⁻¹ after suitable warm-up. The new versions of the spectroMonitor III detector feature a linear sensitivity range of 0.001–2.0 A full scale.

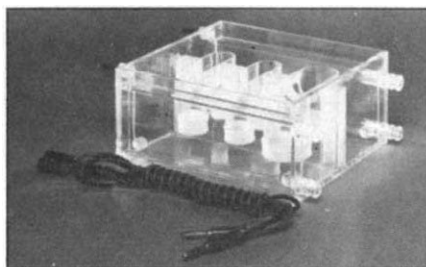
Circle No. 121 on Reader Service Card.

● A new range of pharmaceutical and laboratory autoclaves has been introduced by Edwards High Vacuum. The Edwards Fedegari series FOF autoclaves use the dynamic steam principle of sterilization and have a microcomputer control system dedicated to this sterilization process. There are 24 types of cycle available. Each cycle type must be further programmed by the user with the parameter values required to produce the final programmed cycle.

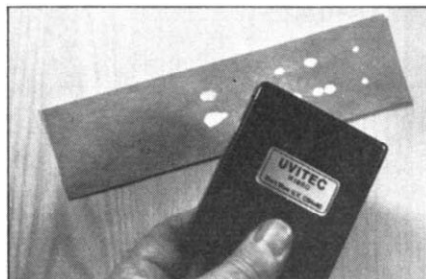
Circle No. 122 on Reader Service Card.

● A new range of UV/visible spectrophotometers, has been introduced by Pye Unicam. The PU8600 series of microprocessor/keypad-operated instruments is headed by two main versions—the PU8600, designed for industrial and teaching applications, and the PU8610 intended for use in the life sciences. A range of new accessories has also been produced.

Circle No. 123 on Reader Service Card.



The electrophoresis system from GRI



Intersci's new UV source

● Genetic Research Instrumentation has developed an electrophoresis system that is designed for the elution of protein samples from gels and the concentration of macromolecules in solution. The concentrated protein may be harvested either as a concentrate solution or bound to specific activity membranes. The system is based on its sample elution wells. These are two cylindrical wells which are bound to each other by a short bridge. The bottom of each well is covered by a membrane. Proteins migrate from one cup to the other under a constant electrical field of 5W within 1 h, either as a solution or bound to the membrane. Up to three samples may be processed simultaneously and two sizes of sample wells are available. The chamber is water-cooled and supplied complete with three sample wells, blank spacers, safety lid and input leads.

Circle No. 124 on Reader Service Card.

● Intersci has introduced a small but powerful UV source that is suitable for examining thin layer chromatography plates. The Uvitec Micro is available in short wave (254 nm) and long wave (365 nm) versions and comes complete with batteries and viewing box.

Circle No. 125 on Reader Service Card.

● Two new Tissue-Tek vacuum infiltration processors are available from Miles Laboratories. These are microprocessor-controlled flow-through tissue processors that automatically perform the tasks of fixation, dehydration, clearing and paraffin-impregnation of tissue. The specimens are safely inside a completely enclosed retort during processing. The processors are available in new 100- and 200-specimen capacity models as well as the original 300-specimen size. They incorporate a control pad which enables the operator to prepare and store up to 10 different processing programs.

Circle No. 126 on Reader Service Card.

● Coulter Electronics has introduced a new multi-parameter discrete chemistry analyser, Dacos. It can analyse any sample, at any time, with a throughput of 450 tests per hour, carrying out single tests, profiles and statistics. It houses a multi-wavelength optical bench, which rotates around a ring of reaction cuvettes to provide continuous absorbance readings at up to eight wavelengths every 8 s. Dacos uses low sample and reagent volumes with a minimum reaction volume of 120 µl. It uses reusable reaction cuvettes to keep consumable costs down. One or two reagents may be added and both sample and reagent blanks are taken during the analysis.

Circle No. 127 on Reader Service Card.



The 1419 balance from Sartorius

● Brinkman Instruments has introduced the Sartorius series 1400 milligram balances. The series includes the model 1405 MP8 (220-g capacity, 0.001-g read ability) and the dual-range models 1412 MP8 and 1419 MP8 (30 to 300-g and 60 to 600-g capacities, respectively, and 0.001 and 0.01-g readability). The quad-range model 1475 MP8 (420-g capacity) features a fine reading of 0.001 g which can be activated anywhere within the weighing range by pushing the tare button. Additional readabilities of 0.002, 0.005 and 0.01 g are automatically displayed at predetermined levels. Additional features include display control of the last digit (always on, always off, or automatically controlled), auto-adjustable display update speed, adjustable digital filter, seven-segment digital display with stabilized readout indicator, automatic electronic calibration and full-range electronic tare. All models come with a stainless steel and glass draft shield.

Circle No. 128 on Reader Service Card.

● The new Elkay XLDG macropipettor with digital capacity selection dispenses volumes from 1 to 5 ml with adjustable 500 µl increments to within $\pm 0.4\%$ reproducibility. It also has a hexagonal body, feather touch plunger action, positive click-stop, digital capacity adjustment and colour-coding to ensure correct tip usage.

Circle No. 129 on Reader Service Card.



The pH meters from Chemtrix

● Wheaton Instruments has introduced the Wheaton Unispense/Omni-Pette, an automatic liquid delivery system for aliquoting as well as aspirating. A feature of the system is the Omni-Pette remote control dispensing head which allows fluids to be delivered at some distance from the pump. The Wheaton Unispense/Omni-Pette will feed, wash and inoculate cells in 90 and 24-well microtest plates when the appropriate microtest manifold is attached to the Omni-Pette. The Wheaton Unispense/Omni-Pette is suited to delivering small volumes of fluid in the 0.1 to 20 ml range. The system is controlled manually by a push button, foot switch or by the flush-mount switch. When programmed in the automatic mode, the Unispense/Omni-Pette will cycle at regular intervals which can also be programmed for 0.5–5 s delay intervals between cycles. A five-digit LED display records the number of doses dispensed, while a cycle alert signals the end of each dispense cycle. *Circle No. 130 on Reader Service Card.*

● Cytogard is the newest addition to Gelman Sciences' laminar flow units. It has been designed to allow the safe handling of cytotoxic drugs. The cabinet, manufactured in compliance with Australian standard AS 2567, the only legislative standard in the world for the handling of cytotoxic drugs, is also designed to satisfy British standard 5726 class II for containment. The construction cabinet has a stainless steel working area. It also includes a pre-filter and final high efficiency particulate air (HEPA) filter for maximum protection and a carbon absorption cell for handling volatile liquids. *Circle No. 131 on Reader Service Card.*

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Wheaton's Unispense/Omni-Pette

● A new family of multi-function pH meters has been produced by Chemtrix. The model 410 pH and mV meter is accurate to ± 0.1 pH and $\pm 0.1\%$ mV units. When temperature is crucial, the model 420 pH/mV meter automatically compensates for sample temperature variations over the 0–100°C range. In addition to this the model 430 pH/mV/temperature meter measures temperature over the 50 to 200°C range with $\pm 0.5^\circ\text{C}$ accuracy. All units have large LCD displays. They are battery powered and operate over the 0–14 pH and $\pm 1,999$ mV ranges. *Circle No. 132 on Reader Service Card.*

● Brinkmann Instruments has released the Epson HX-20 system for Sartorius electronic balances. The system includes an Epson HX-20 computer; an interface for connection to Sartorius MP6, MP7 or MP8 electronic balances and standard programs for statistics, net weight or counting. The HX-20 computer is small enough to fit in a briefcase and requires no power cord. *Circle No. 133 on Reader Service Card.*

Literature and assay kits

● A new test for serum creatinine is now available from J.T. Baker Chemicals. In cooperation with Dutch clinical laboratories, J.T. Baker has developed a creatinine test based on the Jaffé method but free from bilirubin interference. Bilirubin, by use of a special reagent, is oxidized before the reaction rate is measured. The test has been designed for the kinetic measurement of creatinine. Application sheets currently available include Cobas Bio, RA-1000, PA-800 and manual techniques. *Circle No. 136 on Reader Service Card.*

● Boehringer Mannheim Biochemicals now offers an *in vitro* immuno-turbidimetric test kit for the determination of fibronectin levels in human plasma. The turbidity, which results from the antigen-antibody reaction, is measured with a spectrophotometer. The assay uses a standard curve obtained from the set of standards included in the kit. Sample values are compared to this and the plasma fibronectin levels are then determined. The shelf life has been extended and the kit may now be stored at 4°C. *Circle No. 137 on Reader Service Card.*

● Tissue-Tek III uni-cassettes, Tissue-Tek II cassettes and Tissue-Tek embedding rings are now available from Miles Scientific in a selection of colours. Uni-cassettes are available in five new colours: tan, lilac, orange, gold and aqua, in addition to the original five colours: white, pink, green, blue and yellow. The Uni-cassette system also includes a biopsy cassette for small specimens, which is grey. Mega-cassette is available for larger tissue specimens. Tissue-Tek II cassettes are also available in the same 10 basic colours as Uni-cassettes but there is also a clear, polypropylene cassette that allows embedded tissue to be seen. Tissue-Tek embedding rings are available in colours for the first time, these being white, pink, blue, green and yellow. The rings allow larger specimens to be embedded than does Mega-Cassette. *Circle No. 134 on Reader Service Card.*

● Futura II star-series refillable combination electrodes from Beckman are designed to measure pH in difficult situations. These include those in which ionic strength is low, such as in acid rain and drinking water, those where temperature varies substantially or in samples which are sensitive to silver. Star-series electrodes operate in the range of pH 0 to 14 and are accurate to ± 0.01 pH. Response time in the pH 4 to 7 range at constant temperature is stable to ± 0.02 pH in 30 s. For temperature changes from 25°C to 75°C in pH 4 buffer, response is stable to ± 0.02 pH in 30 s. Slope is 97 to 100%, temperature range is 5 to 100°C and isothermal point is 7 pH. *Circle No. 135 on Reader Service Card.*

● A new ^3H RIA kit is now available from Seragen for specific measurement of prostaglandin E_1 (PGE_1). This 100-tube kit contains all the necessary reagents for the test, including antisera specific for PGE_1 which show 9% cross-reaction with PGE_2 . Seragen's new PGE_1 assay is sensitive to 20 pg per 0.1 ml. *Circle No. 138 on Reader Service Card.*

● An instructional brochure is now available from Tago that describes the structure and function of plasma lipoproteins in the transport of cholesterol and triglycerides. Other topics covered in the brochure include the clinical significance of apolipoprotein measurements, Tago's immunoassay systems and population studies using Tago's Diffu-Gen kits. *Circle No. 139 on Reader Service Card.*

● A new brochure is available from Boehringer Mannheim Biochemicals. The brochure contains a complete listing of all molecular biology products, including modifying enzymes, restriction enzymes, linkers and primers, inhibitors, nucleotides and nucleosides. *Circle No. 140 on Reader Service Card.*